MUSCULAR DYSTROPHY IN MICE ON VITAMIN E-DEFICIENT DIET [*]

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It has become evident during recent years that vitamin E is a necessary factor for the preservation of the integrity of skeletal muscle. This has been satisfactorily demonstrated for a number of species: guinea pigs,[1-4] rabbits,[1, 5-7] goats and sheep,[8] rats,[9-11] the tree kangaroo,[12] ducklings[13] and, probably, the dog.[14, 15] Muscular lesions may, under conditions not yet accurately defined, occur also in young chicks upon diets low in vitamin E.[16, 17]

A comprehensive study of the effects of vitamin E deficiency in mice, carried out by Marianne Goettsch,[†] has provided material for a report on the pathology in this species. Little has been previously written on the effects of vitamin E lack in mice, and we have seen no reference to lesions of the skeletal muscles such as will be described in this paper. Indeed, the literature affords very little information as to the requirements of mice for vitamin E. Beard,[18] in 1926, demonstrated that this factor was needed for the successful completion of pregnancy and Bryan and Mason [19] have recently shown that, unlike the rat, the male mouse fails to develop testicular degeneration and sterility on diets low in vitamin E—an observation in complete agreement with our experience. These authors also state that "the neuromuscular disorder so frequently seen during late stages of lactation in the progeny of female rats possessing a critically low storage of vitamin E was not observed in any of our suckling mice."

MATERIAL AND METHODS

The mice were the offspring of mothers maintained through 2 to 7 generations on the following vitamin E-deficient diet:

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[†] This will be published elsewhere.

Diet 1

Casein (technical) 320
Cornstarch 400
Salt mixture 40
Yeast (Fleischmann's brewer's) 100
Lard 220
Cod liver oil 20

During the lactation period, the amount of yeast was doubled. The mothers were given a single dose of alpha-tocopherol or of wheat-germ oil at the beginning of pregnancy. Details of dosage necessary to insure fertility will be published elsewhere by Dr. Goettsch.

Tissues from 293 mice were available for histologic study. Animals which died or were killed on the first or second day after birth were fixed *in toto* for several days in Bouin's fluid. In somewhat older mice, the entire limbs were fixed in Zenker's fluid, and from adult animals, various samples of muscle and viscera were examined individually. Complete study of the central nervous system, after fixation in a 4 per cent solution of formaldehyde, was carried out on a few young and old animals. The following methods were applied: the gallocyanin stain for ganglion cells; Weil's stain for myelin sheaths; Bodian's stain for axons; phosphotungstic acid hematoxylin for possible glial reactions. No systematic study was made of the endocrine organs.

Unlike young rats, which in most cases betray the onset of muscle lesions by dragging their hind limbs and clenching their paws, mice exhibit no recognizable symptoms even when lesions of considerable severity are found. We have not observed a single instance of frank paralysis, and have been unable to predict the lesions while animals were alive. Nevertheless, gross changes in the appearance of the muscle could be detected in some of the more severely affected animals, in the form of yellowish gray streaks.

MICROSCOPIC CHANGES

The material has been arranged on the basis of the age of the mice, since the lesions differ to a certain extent according to age.

Of 22 mice obtained on the first day after birth, 14 were found dead, 8 were killed. However, no animals are included in which the state of preservation, as judged by nuclear staining, was bad.

Edema of the subcutaneous and intramuscular connective tissue, of varying severity, was present in 12 mice, including 3 that were killed, so that it cannot have been due to autolytic change (Figs. 1 and 2). In a few cases, it was accompanied by capillary hemorrhages. *Hyaline*

necrosis of the muscle fibers, sometimes accompanied by pyknosis and fragmentation of the nuclei, was recorded in 7 cases (Fig. 3). The fibers were often of unequal caliber and widely separated by edematous tissue infiltrated with small and large mononuclear cells. In a few instances, polymorphonuclear leukocytes were accumulated about the necrotic fibers. The presence of an inflammatory reaction is definite proof that the degeneration of the fibers is not an artefact or due to trauma or autolysis.

The immature appearance of the muscle fibers, associated with abnormally active blood formation in the liver, suggests that the lesions may have developed toward the end of fetal life rather than *post partum*. However, it is difficult to be certain of this.

The young were from the second to the sixth generation maintained on the vitamin E-deficient diet throughout life. It is not possible to say with assurance that the severity and frequency of the early lesions increases in successive generations, because the number of mice in each generation is too small to make percentages significant. However, in the second and third generations, only 4 of 11, or 36.4 per cent, were affected, whereas in the fourth, fifth and sixth generations, 15 of 27 examined, or 55 per cent, showed lesions—a difference which is perhaps suggestive. This calculation includes only mice dying or killed during the first 5 days.

In this group of 1-day-old mice, edema of the subcutaneous and intramuscular tissue was a more striking feature than necrosis of the muscle fibers. This finding is perhaps analogous to the condition described by Pappenheimer, Goettsch and Jungherr,[16] and subsequently by Dam and Glavind [20] and by Bird and Culton [21] as occurring occasionally in young chicks maintained on a vitamin E-deficient diet. The latter authors have shown that the edema is prevented by wheat-germ oil and by alpha-tocopherol, without other alteration of the diet, so that it may justly be regarded as a manifestation of vitamin E deficiency. Glavind[17] has recently reported the occurrence of muscle degeneration in these edematous chicks; Pappenheimer, Goettsch and Jungherr had also noted lesions of the muscles, and a reëxamination of their material shows that lesions were present in a large proportion of their edematous chicks.

Edema of the muscles is also present during the early stages of the muscular dystrophy of young rats, which occurs at the end of the lactational period. Here, however, it appears to be part of the very violent inflammatory reaction called forth by the sudden necrosis of the fibers and it is often fibrinous in character.

In the next group of 16 mice which died or were killed during the

period from the second to the fifth days inclusive, edema was no longer a feature, and indeed was noted in but one case. Five mice of this group showed hyaline necrosis of the muscle fibers. In none were the lesions very widespread or accompanied by a marked inflammatory reaction or by calcification of the necrotic fibers. In one animal, killed on the fourth day, there was loss of fibers with replacement by young fibrous tissue.

In the third group, comprising 175 mice sacrificed between the 6th and 15th days, the incidence of muscular lesions was much less. Only 17, or 9.7 per cent, showed hyaline necrosis with segmentation into fragments. With one exception, an animal dying on the sixth day, the affected mice were all between the ages of 10 and 15 days. The inflammatory reaction was sometimes slight, sometimes intense (Fig. 4). Calcification of the necrotic fibers was seen in most instances and seemed to follow almost immediately upon the death of the fibers. Often the longitudinal striations were discernible even in the completely calcified muscle fragments.

The fourth group consisted of 32 mice ranging in age from 16 to 35 days. These showed the highest incidence of muscular lesions, namely, 19, or 59 per cent. This age period includes the end of lactation, at which time the young of vitamin E-depleted rats commonly show the most severe dystrophy. The lesions were essentially the same as those occurring in rats, save that the calcification of the necrotic fibers was present in almost every case (Figs. 5 and 6). In one litter killed on the 25th day, only calcified fibers remained; no fresh necrosis or cellular reaction was to be seen (Fig. 7). This was interpreted as complete healing of an earlier previous lesion.

The last group comprised 48 mice killed or dying at various ages from 35 to 439 days. Three of the older rats succumbed to suppurative bronchiectasis and pneumonia. The others showed no abnormal findings apart from the muscular lesions noted on histologic examination. These consisted of isolated necrotic fibers, usually but not always calcified, encapsulated by multinuclear cells or histiocytes (Fig. 8). The great mass of muscle had a normal structure, and it seems evident that the necrotic fibers represent the remains of lesions incurred in early life. In several cases, the calcified fibers were surrounded by adipose tissue, which had obviously replaced degenerated fibers.

The disease thus seems to run its course during the first month of extra-uterine life; and it is not progressive. In this it is comparable to the muscular dystrophy of young rats, which, if the animals survive, undergoes spontaneous cure, even though vitamin E is withheld. The muscular dystrophy of rabbits and guinea pigs, on the other hand, is

a progressive disease often continuing into adult life, and running a chronic course.

A summary of these observations is given in Table I. No alterations of significance were found in the brain, cord or peripheral nerves, nor in the bones or in any of the viscera examined. As in other species, the smooth muscle and myocardium were not affected. In the day-old mice with edema, hematopoiesis in the liver seemed unusually active, but there was a wide individual variation, and we are inclined to regard this as merely an index of delayed development. This is perhaps also true of the muscle fibers, which were of unusually small caliber, had centrally placed nuclei and abundant undifferentiated sarcoplasm.

TABLE I

Age (days)	No. of mice	Lesions	Percentage with lesion
1	22	13	59
2–5	16	5	31
6–15	175	17	9.7
16–35	32	19	59
36–439	48	5	10
Total	293	59	20

As was stated, we can fully confirm the report of Bryan and Mason [19] as to the ineffectiveness of vitamin E deprivation in causing testicular degeneration. Thus five male mice from 221 to 439 days old showed mobile sperm, active spermatogenesis in sections of the testes and fertility on mating.

CONCLUSIONS

1. Female mice maintained on a vitamin E-low diet were given a single dose of wheat-germ oil or of alpha-tocopherol at the beginning of pregnancy to insure the birth of living young.

2. Examination of the skeletal muscles of their offspring showed necrosis of the fibers in about 20 per cent of 293 mice examined.

3. Mice dying or killed on the first day had edema of the subcutaneous and intramuscular tissues; in about one-third of the cases there was hyaline necrosis of the muscle fibers.

4. The incidence of muscular lesions was highest (59 per cent) in a group sacrificed or dying between the ages of 16 and 35 days. There was early calcification of necrotic fibers and active regeneration.

5. Adult mice occasionally showed scattered hyaline or calcified fibers remaining from early lesions, but no progressive dystrophy of the muscles.

6. No lesions of significance were found in the central nervous sys-

tem, or in other organs or tissues. In mice, spermatogenesis was active on the vitamin E-deficient diet up to 439 days.

NOTE: We wish to acknowledge our indebtedness to Claudia Schogoleff for her assistance in carrying out the experiments and to express our thanks to Dr. Abner Wolf for reviewing the sections of the central nervous system. Dr. R. D. Shaner, of Hoffmann-LaRoche, Inc., very kindly supplied the alpha-tocopherol used in this work.

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DESCRIPTION OF PLATES

PLATE 23

FIG. 1. Mouse no. 122a. Died on first day. Marked subcutaneous and intramuscular edema. Swollen hyaline fibers with pyknotic nuclei. × 95.

FIG. 2. Mouse no. 84a. Killed on third day. Normal skin and muscle. × 95.

FIG. 3. Mouse no. 122a. Died on first day. Edema; separation and hyaline necrosis of fibers; capillary hemorrhages. Cellular reaction. × 110.
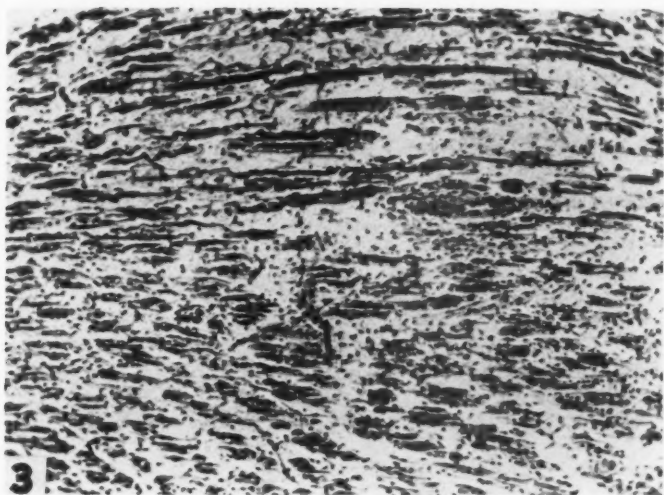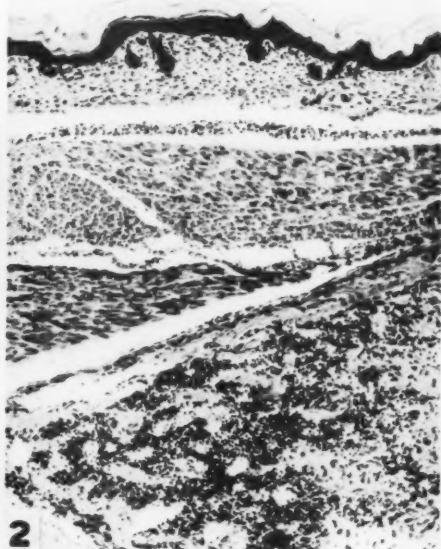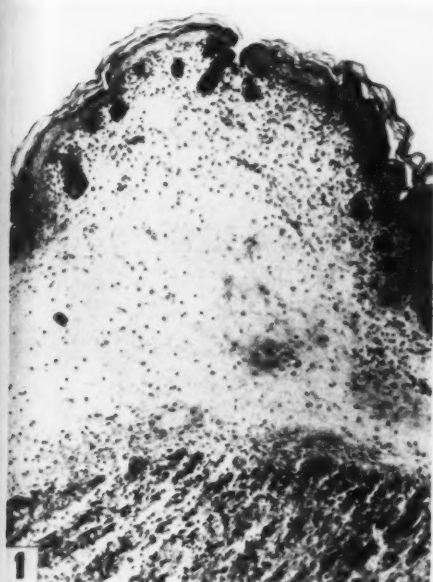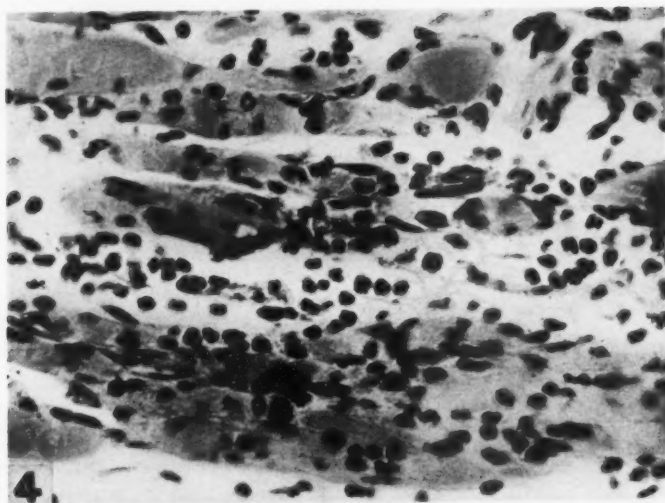
Pappenheimer

Muscular Dystrophy and Vitamin E Deficiency

PLATE 24

FIG. 4. Mouse no. 277a. Killed on eleventh day. Hyaline necrosis of fibers with polymorphonuclear reaction. × 460.

FIG. 5. Mouse no. 110b. Killed on 16th day. Extreme necrosis of fibers with calcification. × 110.
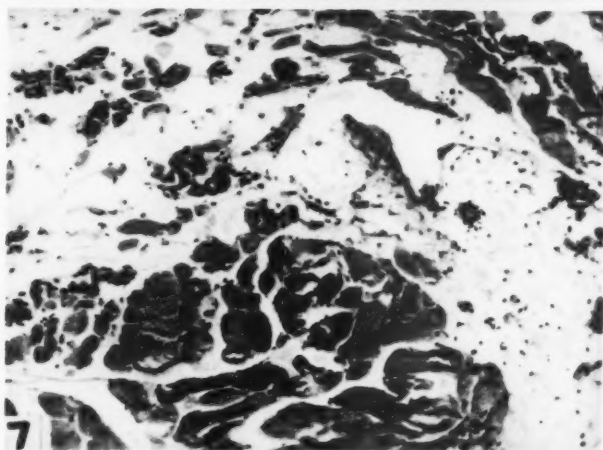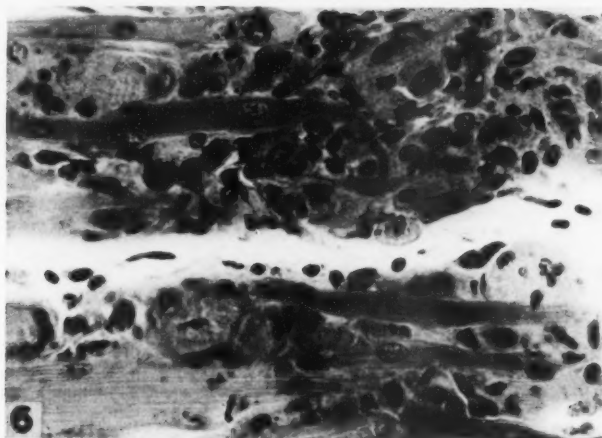
Pappenheimer   Muscular Dystrophy and Vitamin E Deficiency

PLATE 25

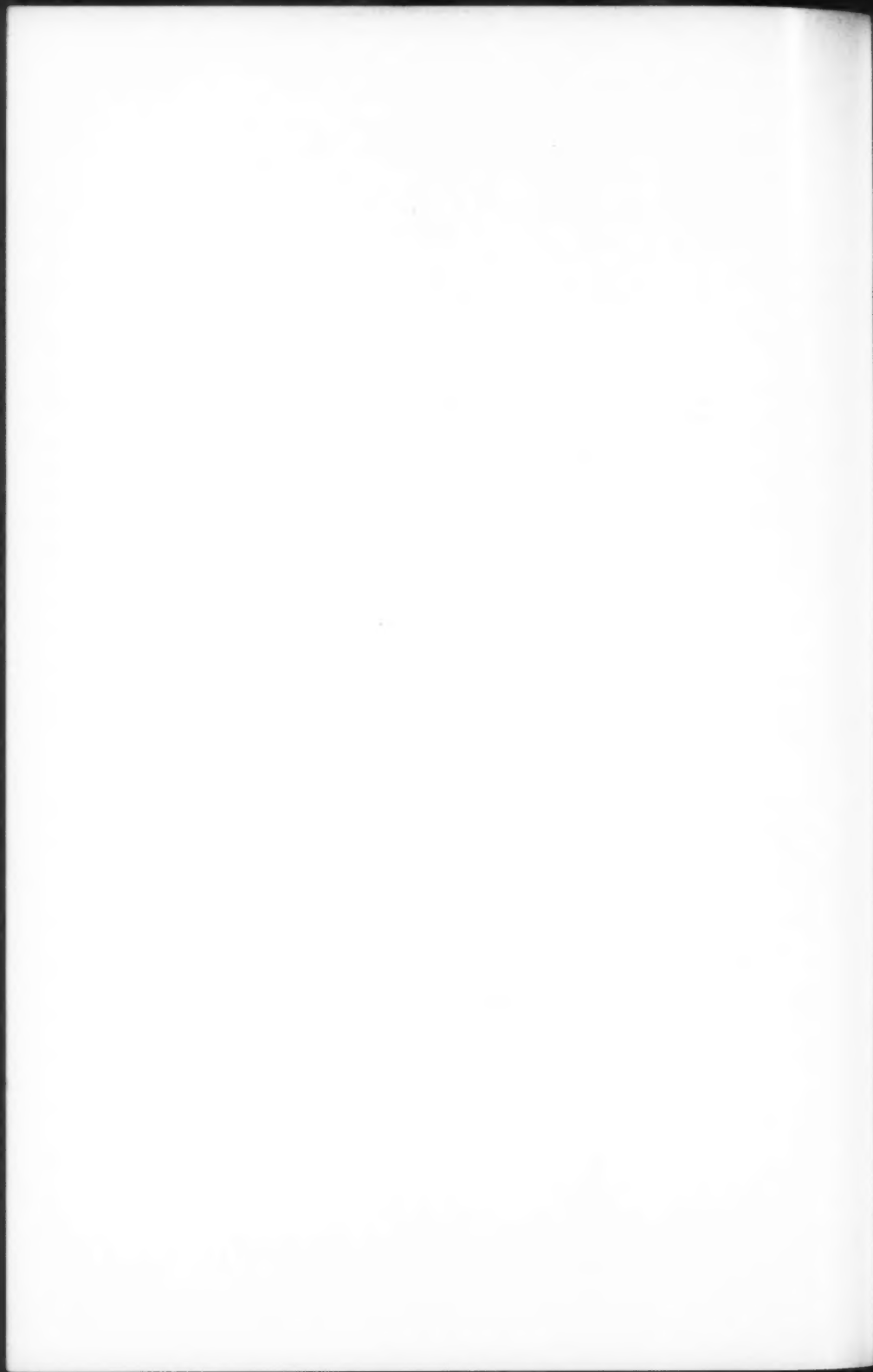FIG. 6. Mouse no. 122d. Killed on 24th day. Necrosis of fibers; activation of myocytes; early regeneration. × 414.

FIG. 7. Mouse no. 192e. Killed on 25th day. Calcified fibers surrounded by adipose tissue. No reaction. Healed lesions. × 99.

FIG. 8. Mouse no. 310. Killed on 123rd day. Old calcified fibers. × 414.

THE GLOMUS TUMOR *

Investigation of Its Distribution and Behavior, and the Identity of Its "Epithelioid" Cell

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The glomus tumor is reputed to be an enlarged caricature of the highly specialized glomic arteriovenous anastomoses which have been found only in certain parts of the hands and feet at the cutaneous-subcutaneous junction. Is it really true, as reported, that glomus tumors occur elsewhere in the body, and if so how can this be explained? Are glomus tumors always small encapsulated neoplasms or do they ever display infiltrative growth or metastasize? What is the nature of the "epithelioid" cells which are so characteristic of the glomus tumors? Certain observations have come to our attention which throw some light on these phases of glomus tumors. We propose to discuss them in the order given.

Distribution of Glomus Tumors

In 1935, Masson published a monograph entitled "Les glomus cutanés de l'Homme." In this he described the normal glomuses of the hands and feet and stated that his personal experience with glomus tumors included 27 cases: 14 in the fingers and hands, none in the feet and 13 in other parts of the upper and lower extremities. All of these involved cutaneous and subcutaneous tissues. He stated that their occurrence in places where normal glomuses have never been found might be explained in one of two ways: either glomuses exist in these areas but are so widely scattered that the chance of biopsies has never permitted their discovery, or else such glomus tumors come from heterotopic glomuses.

A survey of 240 cases reported in 89 different publications, including 33 personally observed cases, indicates that the distribution is much wider than this and that the tumors can arise in other zones beside the cutaneous-subcutaneous junction. Three tumors have been found in the face. Two of these were reported by Butz (1940) and one in the eyelid was studied in this laboratory and reported by Kirby (1941). Grauer and Burt (1939) found two on the penis; Gumpel (1939), one in the axilla; Roger and Alliez (1938), one on the lateral thorax in the region of the twelfth rib; Touraine, Solente and Renault (1936) and

Schulte and Isselstein (1935), several tumors scattered over the trunk; Lendrum and Mackey (1939) reported one on the buttocks (we also have observed one in this region), and Kirchberg (1936) reported one in the neck and another from the back. Fernández and Monserrat (1931) and Sannicandro (1936) reported finding them in the auricle but there is doubt whether these are true glomus tumors. Kolaczek (1875), Buzzi (1887), v. Hleb-Koszańska (1904) and Kofler (1936) have each reported a glomus tumor of Luschka's coccygeal glomus.

The evidence that glomus tumors can arise away from the cutaneous-subcutaneous junction and from Luschka's glomus is scanty but convincing. André-Thomas (1933) described a patient with two glomus tumors: one within the substance of the vastus internus muscle and the other in the region of the internal condyle of the femur adherent to the aponeurosis of the crureus muscle where it fuses with the vastus internus. These were accepted as glomus tumors by Oberling and Roussy and mentioned in 1935 by Masson. Hoffmann and Ghormley 7 (1941) found a characteristic glomus tumor within the capsule of the knee joint. They were kind enough to permit us to examine a section and there can be no doubt about the diagnosis. The multiple tumors described by Bergstrand (1937) included one near the sheath of the peroneal tendon, another subfascial near the lateral malleolus, a third behind the malleolus in a hollow in the talus. Swenson's (1940) patient had a glomus tumor of the palmar surface of the left middle finger which lay deep to the fat pad almost on the periosteum. In Kulenkampff and Heilmann's (1940) case the glomus tumor thickened the wall of a vein which ran along the abductor pollicis longus and brevis muscles. We have studied a similar case in which the tumor surrounded a subcutaneous vein situated on the dorsal aspect of the base of the thumb. Iglesias de la Torre, Gomez-Camejo and Palacios (1939) have described a tumor of characteristic symptoms and morphology which lay buried within the terminal phalanx without any involvement of the tissues outside of it. The cases of Durante and Lemeland (1928) in the uterus and of Kirshbaum and Teitelman (1939) in the omentum are too questionable to be considered here.

The evidence just cited seems sufficient to warrant the belief that glomus tumors can develop not only in the junction of skin and subcutaneous tissue in regions of the body where no glomuses have ever been observed but they can develop also in deeper structures.

Infiltrative Growth, Recurrence and Metastasis

Almost all observers of glomus tumors have been impressed by the fact that they are simply enlarged, encapsulated caricatures of the normal glomus and that they are easily removed and do not recur.

Nevertheless, locally recurring tumors have been reported by Lewis and Geschickter (1935) in the forearm, by Meyers (1939) in the thigh and by Kirby (1941) in the eyelid, and we have studied three other recurrent cases beside that of Kirby—one in the knee region, a subungual tumor of the finger and the third in the distal anterior closed space of a finger. It need not be supposed that these recurrent cases prove infiltrative growth, since they may be explained either as incomplete excisions or as the formation of new glomus tumors. It is now well established that individual patients may have multiple glomus tumors: Adair (1934), André-Thomas (1933), Bergstrand (1937), Davies, Hellier and Klaber (1939), Hval and Melsom (1936), Schulte and Isselstein (1935), Stout (1935), Touraine, Solente and Renault (1936), Weidman and Wise (1937).

So far as we are aware, there have been reports of only two tumors which have received the label of malignant glomus tumor. One was described by Kirshbaum and Teitelman (1939). It arose seemingly in the omentum, invaded the stomach and metastasized to the liver, and because it consisted of rounded cells collected into many layers about vascular endothelial-lined tubes, was called by the late R. H. Jaffé a malignant glomus tumor. The other case, a tumor of the pectoral region, was reported by Soiland (1937), and the reasons for calling it a glomus tumor do not appear in the text or illustrations. It seems to us that neither of these cases can be accorded unqualified acceptance as a glomus tumor.

Report of Case. Through the kindness of Dr. C. Zent Garber, pathologist of the New York Orthopaedic Hospital, we have had the privilege of studying a progressively infiltrating vascular tumor which we have good reason to believe is an authentic glomus tumor.

The patient was an Italian male. In December, 1927, at the age of 14, he began to have sharp pains below the right internal malleolus on weight-bearing, and also some tenderness. When examined a year later he had a slight foot drop, the foot was cool and damp and there was a vague soft tissue mass internal to the os calcis in the region of the calcaneo-astragaloid joint. The mass extended downward during the next 8 months and an abnormal pulsation was felt over it, with a rusty discoloration of the skin. A tumor measuring 5 by 2.5 cm., of indefinite outlines, was excised in August, 1929. It lay caudad to the posterior tibial vessels and nerves and did not appear grossly vascular. There was evidence of recurrence 2½ years later. The patient refused further treatment and was not seen until November 7, 1940, more than 11 years after the first operation, when he returned because of suddenly developed pain and tenderness in the tumor. Examination now showed extension of the tumor anterior to the malleolus onto the dorsum of the foot and also upward posterior to the ankle. There was marked atrophy of the leg and foot muscles, the foot was cold and blanched, it hung at 110° equinus and motion was possible only from 110° to 95°. A second attempt was made to excise the tumor on December 2, 1940. It extended widely in the tissues covering the medial and dorsal aspects of the tarsal bones, the medial malleolus and below it and behind the ankle joint. It was adherent to the posterior tibial nerve and artery and the

dorsalis pedis artery. As this operation obviously failed to remove all of the neo-
plastic tissue and the remaining tumor continued to grow, causing ulceration, an
amputation was performed on February 5, 1941, through the upper third of the
leg. The tumor now had extended 3 cm. above the insertion of the Achilles tendon
which was surrounded by it.

Microscopically this tumor proved to be a vascular neoplasm of an
infiltrative type. At its advancing margin the vessels which sometimes
contained erythrocytes were simple tubes of endothelium often sur-
rounded by collagen sheaths (Fig. 1A). Very quickly, however, rounded
cells appeared in the vessel wall attached to the outer aspect of the
tube. These surrounded the vessel in layers, as a rule from one to five
cells thick (Figs. 1A and 1B). The cells in every way resembled the
epithelioid cells of the glomus tumor. They were rounded with a cen-
trally placed hyperchromatic nucleus, often a clear zone about the
nucleus, and many of the cells were separated from their neighbors by
delicate reticulin fibers. Occasionally the tumor cells with their nuclei
were elongated and sausage-shaped, but this was uncommon. The
sheathed vessels appeared scattered throughout the invaded tissues,
which usually were not destroyed by them. At infrequent intervals
tumor nodules were formed consisting of a tightly packed mass of
sheathed vessels so arranged that the epithelioid cells of one vessel were
in continuity with those of its neighbors and the nodule appeared like
a solid mass of epithelioid cells with endothelial tubes scattered through
it (Fig. 1C). The tumor vessels hardly ever formed straight tubes but
twisted and turned sharply so that they were usually seen in cross
and tangential sections. The tumor vessels were in continuity with
arteries and veins but in none of the sections examined could any
smooth muscle be demonstrated in the walls of tumor vessels nor could
myofibrils be found in the epithelioid cells. None of the tissue was
fixed in chloral hydrate so that no satisfactory neurite stains were ob-
tained and we can furnish no information about this feature.

On histological grounds alone there is good reason for believing that
this is an infiltrating glomus tumor. But further evidence in favor of
this interpretation has been obtained from the study of tissue cultures
made from this tumor and their comparison with cultures previously
obtained from two ordinary glomus tumors. The study of these cultures
has revealed some interesting facts about the "epithelioid" cells which
we believe throw some light on their nature.

CHARACTERISTICS OF THE GLOMUS TUMOR IN VITRO
Infiltrated Area

Cultures made from the external, infiltrative zone of this tumor,
which is composed almost entirely of meandering capillaries, produce
an outgrowth of fibroblast-like cells. Often these form a membranous

sheet which, when treated with silver nitrate, shows sharply stained cement borders around cells in mosaic arrangement. The nuclei of these cells are greatly flattened, as is frequently the case with endothelium, rather poor in chromatin, and surrounded by a few perinuclear granules and a wide and greatly attenuated ectoplasmic zone (Fig. 10). Such a membrane usually grows over the surface of the clot, and often covers a stratum of spindle-shaped or fan-shaped fibroblasts which push their way through the clot and do not take the silver nitrate stain.

Tumor Nodules

When a tumor nodule composed mainly of epithelioid cells of the glomus type is cultivated *in vitro*, the so-called epithelioid cells show themselves to be far from epithelioid. Their habit is discrete, their cell-body is not voluminous, and they have many ramose processes. The progress and cellular constituents of such a culture handled by the Maximow method, are as follows:

1. During the first 24 hours macrophages and small lymphocytes make their appearance, as in cultures from the infiltrative zone. Some mast cells are present in the explant.

2. Within 48 hours there is to be seen at the edge of the explant a corona of fine hairlike pseudopodia leading out small cells with bulging nuclei. The compact nodule is observed to dissolve gradually into these small, branched, discrete cells which within a week have formed a reticular zone extending some distance out beyond the main body of the explant (Fig. 3). The reticular appearance of this zone is produced by the relatively uniform distance between the cell bodies, and by the frequent overlapping of their filamentous but branched processes. These processes do not actually unite, but are extended through the clot at different levels, so that no syncytial structure is formed. Because of these extensions in three dimensions, photography is rendered difficult. The cells under discussion bear no relationship to macrophages or monocytes, from which they can readily be distinguished by their morphology and staining reactions (Figs. 3 and 6). They are entirely unaffected by neutral red and lithium carmine, which are taken up readily by the macrophage and the monocyte. Because of their position with respect to the blood vessels of the tumor, and for other reasons which will be set forth later on, the cells emigrating from these "epithelioid" nodules will henceforth be referred to as "pericytes."

3. Mingled with the pericytes are a few fibroblast-like cells, and at the periphery of the pericyte zone are varying numbers of broad, flattened fibrocytes with numerous fine processes. Their origin is obscure; possibly they may stem from the macrophages, or possibly from the stroma of the tumor (Fig. 5-F).

4. In 10 or 12 days, capillary buds begin to push through from the explant into the zone of migrating pericytes. They advance at first as solid spikes, but later show themselves to be hollow tubes behind the foremost cells, with lumina sometimes containing blood cells (Figs. 2 and 5).

5. From the 12th day, when they appeared, to the 30th day (when cultivation was terminated), the capillary buds progressively became encrusted with pericytes. These begin to attach themselves to the capillaries as soon as the latter appear, and at some points pile up two deep. They are readily distinguishable from the endothelial cells by their smaller size and branching shape, and especially by their globular nucleus with its one or two nucleoli and richer chromatin network, as compared to the oval, paler nucleus of the endothelial cell with its larger complement of nucleoli (Figs. 2 and 5). The pericytes are indifferent to macrophages and to cotton fibers or other fortuitous inclusions in the medium, and probably also to fibroblasts in their flattened or fan-shaped form. The behavior of these cells *in vitro*, as well as their position encircling blood vessels in the sections, indicate that they are of the nature of vascular satellites. The ramose processes which characterize the pericytes are sometimes exceedingly delicate, so that they are hardly made visible by ordinary stains. But if an intensive staining method, such as silver impregnation, is used, much is brought to light that may not otherwise be seen. Compare Figure 7 made after prolonged treatment by the Bodian method for axis cylinders, with Figure 6 from a preparation stained with Mallory's phosphotungstic acid and hematoxylin. The fields are comparable and the magnifications equal. Nuclear inclusions in the form of clear, non-staining vacuoles are common among these cells, in both sections and cultures.

In addition to the infiltrative glomus tumor described above, we have cultivated two benign glomus tumors. In the latter there was not as great an amount of migration from the epithelioid areas as in the former, and the cultures did not produce encrusted capillaries. But in both, the characteristic cell composing the epithelioid trabeculae was shown to be the pericyte, discrete and branching (Fig. 8). We were not using silver impregnation methods on this tissue at the time of cultivating these benign glomus tumors, and so did not demonstrate any of the fine ramifications.

Masson, discussing the epithelioid areas in his descriptions of the normal and pathological glomus, stated that these cells form a syncytial network, interspersed by a framework of reticulin and elastin, and that they contain varying numbers of myofibrils, which sometimes extend from one cell to its neighbor, by way of their protoplasmic anastomoses.

Myofibrils are entirely absent from some of the glomus cells. According to Bailey (1935), there are no myofibrils in the glomus cells and only occasionally are groups of two or three nuclei seen without demonstrable cell boundaries.

In our cultures no reticulin was formed around the pericytes, though this was present in the sections. No myofibrils were observed in either sections or cultures of these tumors. In the cultures a few binucleate cells were seen, but, as has been stated previously, the cells were otherwise discrete and tended to range themselves at a distance from one another wherever there was room, except when in contact with a capillary, or with another pericyte upon a capillary. There seems to be a possibility that the processes of one cell passing over or wrapping around those of its neighbor in a compressed tumor nodule might produce the appearance of a syncytium such as Masson described.

DISCUSSION

Identification of the "Epithelioid" Cell of the Glomus Tumor as the Capillary Pericyte of Zimmermann

Students of the *normal* glomus agree that the characteristic cells enveloping this short-circuiting vessel between artery and vein pass through a regular series of transitional forms, from the typical spindle-shaped smooth muscle cell of the artery to the epithelioid cell, and on across the bridge to the smooth muscle cell of the vein. In the epithelioid cells, myofibrils are reputed to be sparse or absent, though they are numerous in the coverings of artery and vein. Nevertheless, because of the function which these cells perform in occluding the glomus vessel, as well as because of the gradations observed, they are generally regarded as modified smooth muscle cells.

The characteristic cell of the glomus *tumor* appears to be the epithelioid cell at the peak of its divergence from the smooth muscle of vessel walls. Though Masson has repeatedly reported the presence of myofibrils in the pathological glomus, the majority of other observers have not. Nevertheless a contractile function seems to be indicated for these cells in the pathological as well as in the normal glomus.

The spatial and morphological transition in the glomus, from smooth muscle to epithelioid cell and back again, finds a significant parallel in the investiture of capillaries in various organs, as described by Zimmermann in 1923. This comprehensive paper (Der feinere Bau der Blutcapillaren), dealing with all the classes of vertebrates as well as with man, sets forth the thesis that "in vertebrates in general the blood capillaries are wrapped around by a special kind of cell which in both directions—towards the arteries and towards the veins—passes over

gradually, through transitional forms, into smooth muscle fibers." To this cell, together with its transitional forms, he gives the name of "pericyte." Though they do not contain myofibrils, he regards them as contractile cells, which exercise a mild regulatory function upon the blood flow through the capillaries of various organs. In man, pericytes have been demonstrated in the kidney, the liver, the heart (where they are especially numerous), the lung, the gut, the tongue and the pia mater. They were not found in the spleen. Zimmermann did not investigate the skin or the extremities.

The pericytes of Zimmermann have a compact, usually rounded nucleus surrounded by a variable amount of cytoplasm from which extend exceedingly long, branching runners which embrace the capillary and sometimes extend from one capillary to another (Fig. 5B). These processes are so thin that they can be demonstrated only by some intensive and selective staining method; for this purpose Zimmermann employed variants of the Kopsch modification of Golgi's silver impregnation technic for nerve cells. The branching processes of the pericyte grasp the capillary wall tightly and adhere so closely that no solution can be forced between. According to Zimmermann, only the secondary branches contract and not the main runner which extends lengthwise along the vessel.

These pericytes are analogous in position to the epithelioid cells of the glomus, and in function also, if Zimmermann's conclusions are correct. The apparent difference in form between pericyte and glomus epithelioid cell obscured this relationship until cultivation *in vitro* brought to light the true form of the epithelioid cell. When our cultures were treated by a modification of Bodian's silver method and compared with Zimmermann's figures of pericytes treated by the Golgi method, there emerged a strong presumption that the two kinds of cell were identical (Figs. 7 and 4). Zimmermann's investigations of pericytes extended over some 40 years and were recorded in hundreds of figures. When these are compared with the epithelioid cells which our tissue cultures make available for study, there appears little doubt that we are dealing with the same type of cell, displaying its very delicate ramifications where the population is sparse, as on the capillaries, and *in vitro*, but having them obscured where there is crowding, as in the normal and pathological glomus nodules.

Our material throws no light on the function of the epithelioid cell or pericyte. These cells were never observed to contract spontaneously *in vitro*, as we have seen striated muscle and occasionally smooth muscle contract. They were not stimulated mechanically or electrically. In the early stages of their emigration from the tumor nodules they

were often unipolar or bipolar and spindle-shaped (Fig. 9), becoming ramose later. It is impossible to say whether their processes, once extended, contracted or were withdrawn over a long period of time; but there was no sudden change in form. There was no evidence of secretion, or of their functioning as *Quellzellen* (Schumacher, 1939). It should be borne in mind, however, that we have been dealing entirely with neoplastic cells, not with normal cells, and that they had no nervous connections.

The question naturally arises whether the pericyte is identical with the adventitial cell of mammalian capillaries, whose function and relationships have been the subject of much recent investigation. Zimmermann stated emphatically that the pericytes have nothing to do with the adventitial cells of the veins and arteries, which are fixed stellate connective tissue cells. They are distinct from the Kuppfer cells of the bile capillaries. But since Zimmermann did not investigate the skin of the extremities, it is not possible to compare his data directly with the observations of Clark and Clark (1940) upon the blood vessels of the rabbit's ear.

From their observation of the living "extra-endothelial cells" of blood vessels by means of a transparent chamber in the ear, Clark and Clark concluded that the adventitial cells of the capillaries in this location develop from connective tissue cells in the vicinity. The longitudinally arranged adventitial cells on capillaries and small venules show no evidence of contractility. But as a capillary is transformed into an arteriole, these cells multiply and re-orient themselves transversely, becoming smooth muscle cells and contracting if they are reached by a regenerating vasomotor nerve.

In spite of the relationship of these adventitial cells to smooth muscle, there is little in Clark and Clark's figures or description to indicate that they were dealing with pericytes. In their living, unstained material they should have been able to see numerous branched processes as we see them on the pericytes in living cultures, if such processes were present on the adventitial cells. Indeed, while maintaining the general thesis that the adventitial cells of both amphibian and mammalian capillaries are inert and that the blood flow at the periphery is regulated by the smooth muscle cells of the larger vessels, Clark and Clark agreed that their evidence should not be applied to other forms and to other regions. In this connection they cited the work of Rouget (1873), Vimtrup (1922, 1923), and others on the nictitating and hyaloid membranes of amphibians, in which it appears that certain pronged adventitial cells (the Rouget cells) do exercise a contractile function and represent a primitive form of smooth muscle cell.

Two studies of normal blood vessels in tissue culture have come to our attention which suggest that the writers might have been dealing with pericytes; though this is not altogether clear, since they did not employ silver-impregnation methods. Herzog and Schopper (1931), cultivating the pia mater of guinea pigs, provided several suggestive figures; and Scriba (1935), in a study of circulatory growth in the 9-day chicken embryo, stated that adventitial cells appear in two forms: sometimes as large mesenchyme cells of fibroblastic type, and sometimes in a form resembling macrophages. A further study of such vascular satellites might prove profitable.

It seems likely that in the epithelioid cell of the glomus and in the pericyte which Zimmermann has demonstrated upon the capillaries of certain organs (and Schumacher has described on certain thyroid vessels and elsewhere), we are dealing with a type of cell which, though related to the common adventitial cell as investigated by Clark and Clark and others, is, in its differentiated form, distinct. It may possess contractility to a greater or lesser degree; but its form alone entitles it to separate consideration.

The identification of the so-called epithelioid cell of the glomus tumor as a pericyte has led us to review some of the other vascular tumors with cells arranged peripherally about the vessel wall, which are at our disposal. There are a number which on morphological grounds cannot be classified as glomus tumors and which on the other hand do not correspond with any of the accepted classes of vascular tumors. We believe that their cells may be classified either as pericytes or as adventitial cells and we propose to discuss these tumors in a future communication.

SUMMARY

Evidence has been adduced which provides information in answer to the three queries propounded at the beginning of this paper. Glomus tumors can form not only in the cutaneous-subcutaneous zone of those parts of the body where no normal glomuses have been identified but also in deeper tissues such as joint capsule and striated muscle. A glomus tumor which displayed progressive infiltrative growth has been described indicating that not all of these tumors are localized and encapsulated. However, we do not believe that sufficient evidence exists to establish the fact of metastasis. The "epithelioid" cell of the glomus tumor has been identified as the pericyte of Zimmermann. Since this cell has been demonstrated in many parts of the body, this identification offers a satisfactory explanation for the occurrence of glomus

tumors in those regions of the body where normal glomuses have never been found.

NOTE: We wish to acknowledge our indebtedness to Miss Irene Bokoff for her technical assistance in the preparation of the tissue cultures.

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DESCRIPTION OF PLATES

PLATE 26

FIG. 1. Photomicrographs of infiltrating glomus tumor.
A. The peripheral zone with sprouted capillaries. Some capillaries are simple
    endothelial-lined tubes with a collagenous sheath; others have "epithelioid"
    cells (pericytes) adherent to the sheath.
B. Endothelial-lined tumor vessels surrounded by several layers of "epitheli-
    oid" cells (pericytes) with sharp cell margins and clear zones around their
    nuclei. There are reticulin fibers between the pericytes.
C. A circumscribed tumor nodule of collapsed endothelial tubes surrounded by
    massed "epithelioid" cells (pericytes). One of them shows a nuclear vacuole.
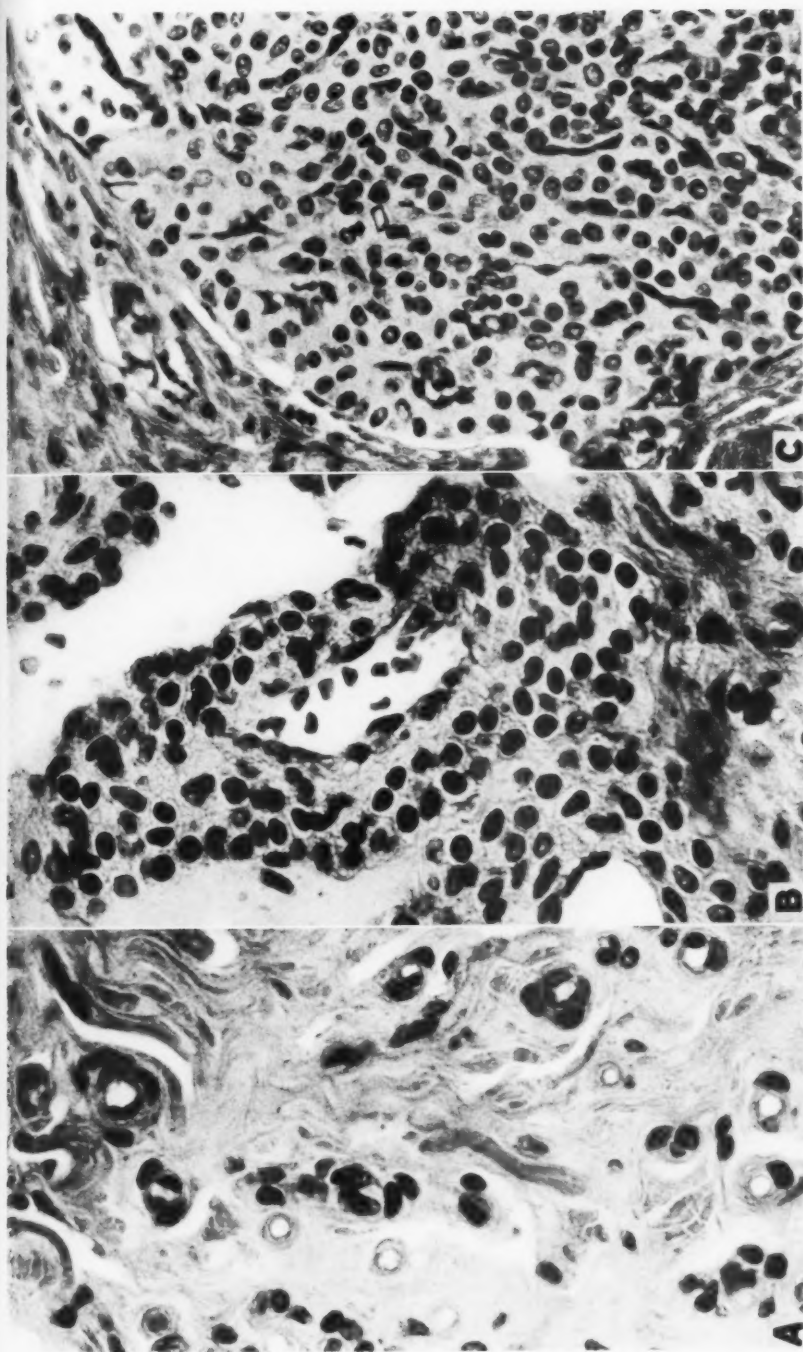
FIG. 2.   A. Tissue culture from "epithelioid" nodule. Twenty days *in vitro;* Zenker's
fixation, phosphotungstic acid hematoxylin stain.

B. Similar material. Twenty-eight days *in vitro.* Kopsch fixative, phospho-
tungstic acid hematoxylin stain. E, endothelial nucleus; P, pericyte nucleus.

FIG. 3.   A. Tissue culture from tumor nodule composed mainly of "epithelioid" cells.
Ten days *in vitro;* Zenker's fixation, phosphotungstic acid hematoxylin stain.
C, capillary bud; I, nuclear inclusion body; M, macrophage; P, pericyte or
"epithelioid" cell.

B. Similar material, 24 days *in vitro,* 4 per cent formaldehyde solution, Bodian
silver impregnation.
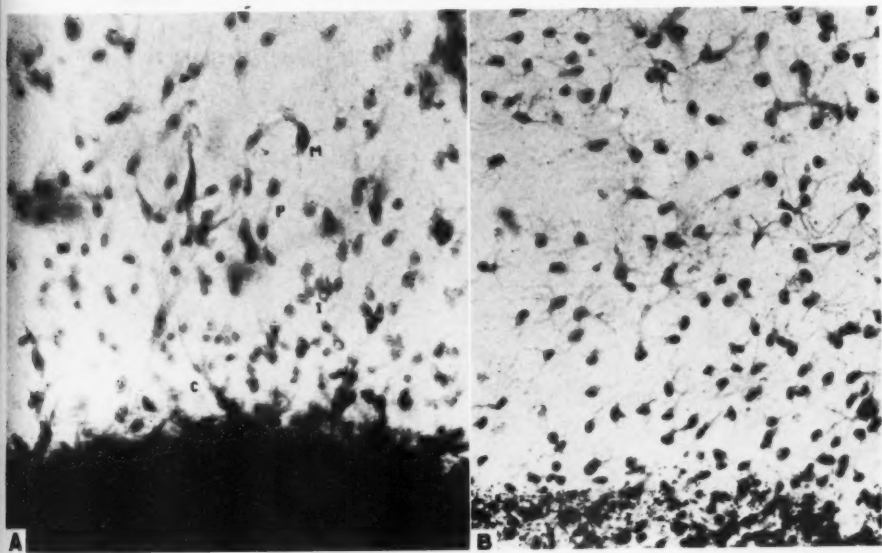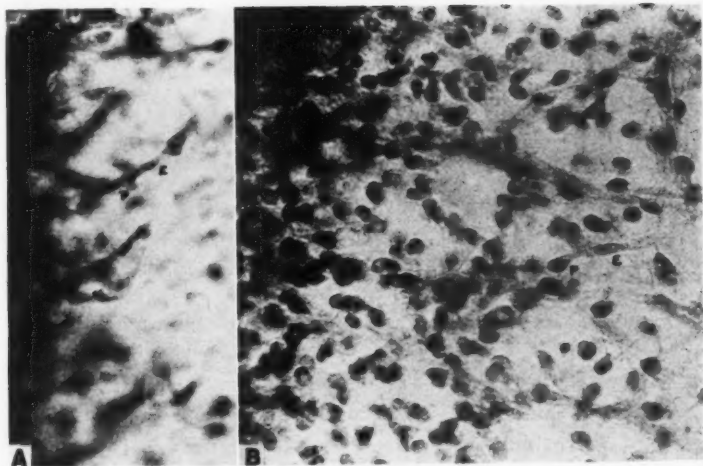
PLATE 27

Murray and Stout

3

Glomus Tumor

FIG. 4. A. Redrawn from Zimmermann (1923). Capillary pericyte with secondary processes contracted; from heart of 43-year-old man.

B. Pericytes from "epithelioid" nodule of infiltrating glomus tumor (18780). Twenty-four days *in vitro*, Bodian silver impregnation.

FIG. 5. A. Tissue culture from "epithelioid" tumor nodule. Twenty-eight days *in vitro;* Kopsch fixative, toluidine blue stain. E, endothelial cell; F, fibrocyte; P, pericyte; R, red blood cell.

B. Redrawn from Zimmermann (1923). Capillary and precapillary pericytes, in the heart of a 43-year-old man.
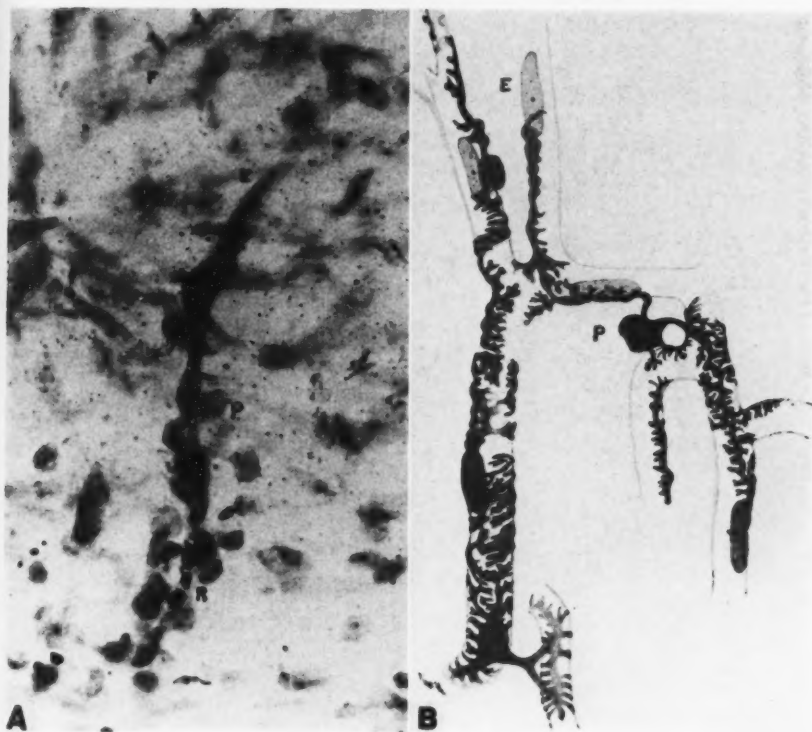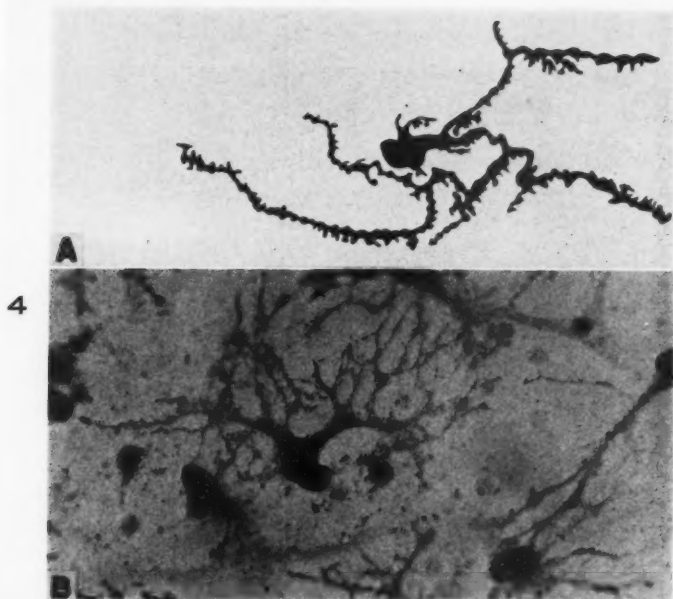
PLATE 28
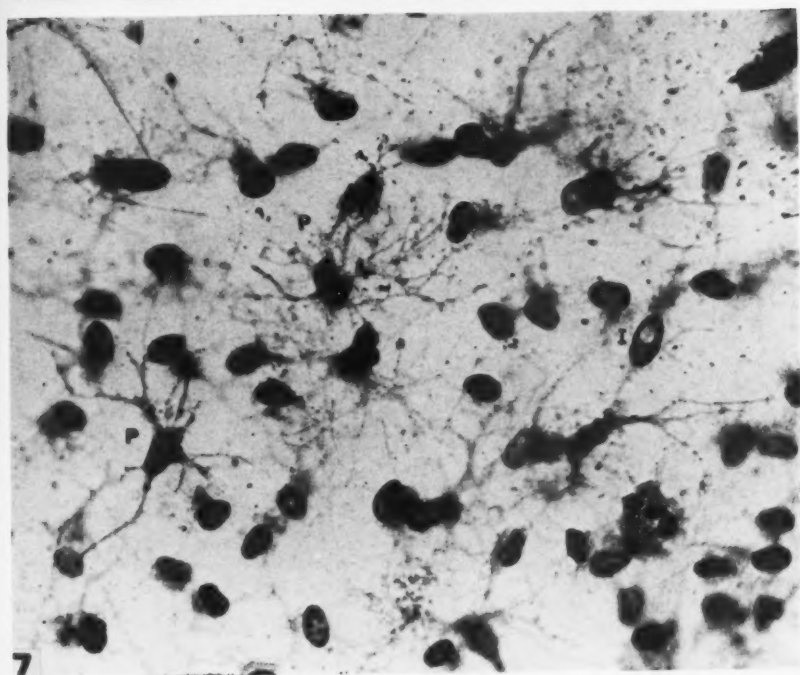
Glomus Tumor
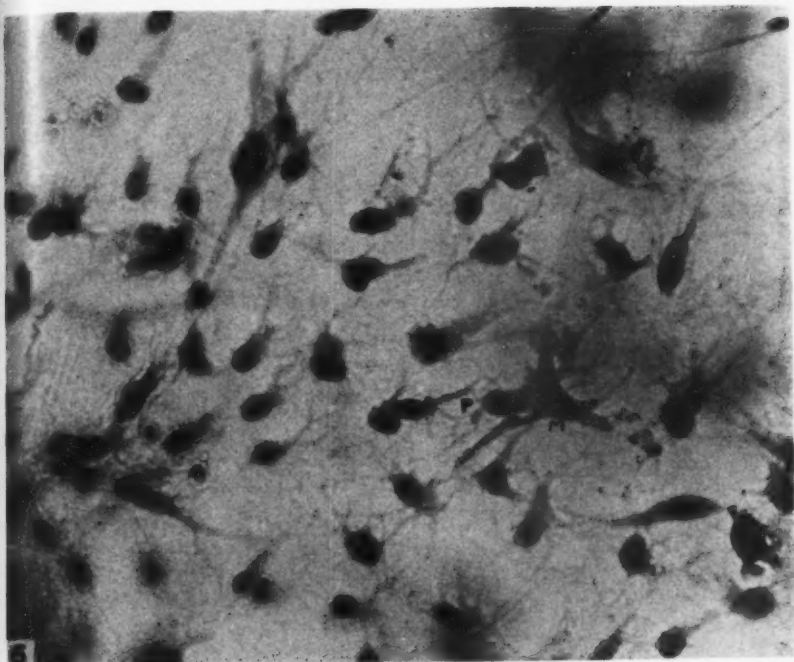
FIG. 8. Tissue culture from a benign, circumscribed glomus tumor from nail-bed of finger (72078). Six days *in vitro;* Zenker's fixation, phosphotungstic acid hematoxylin stain.

FIG. 9. Tissue culture from infiltrating glomus tumor (18780), "epithelioid" nodule; showing pericytes in monopolar and bipolar spindle-shaped form. Five days *in vitro;* Zenker's fixation, phosphotungstic acid hematoxylin stain.

FIG. 10. Tissue culture from external zone of infiltrating glomus tumor (18780). Twenty days *in vitro;* silver nitrate, toluidine blue.
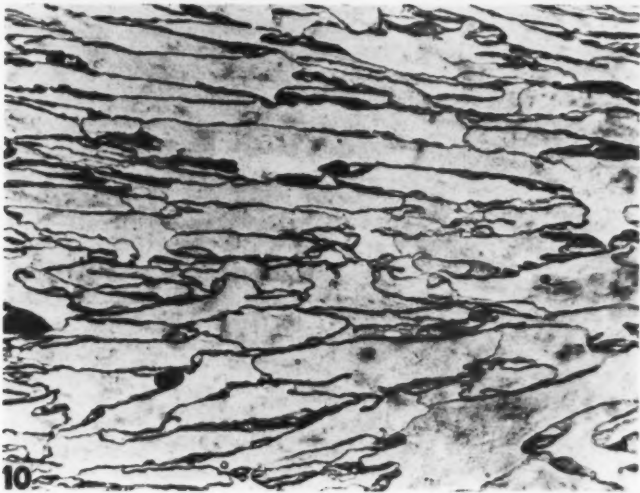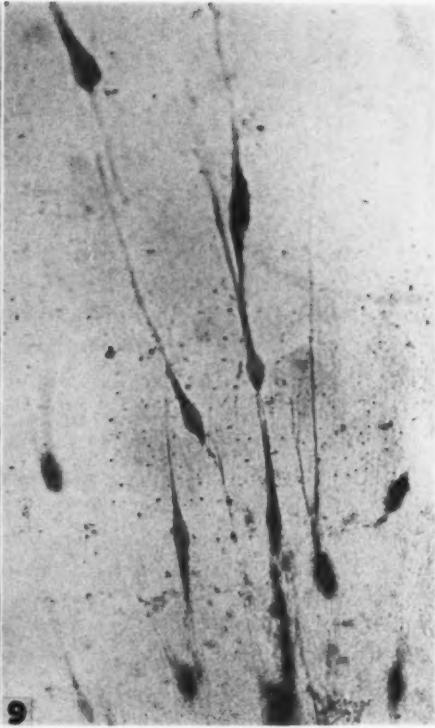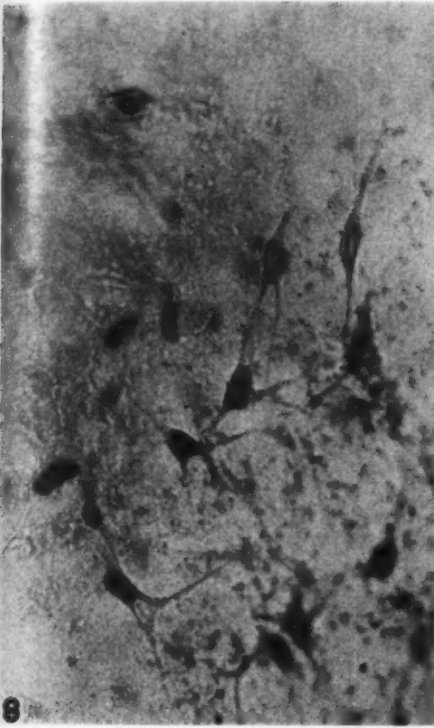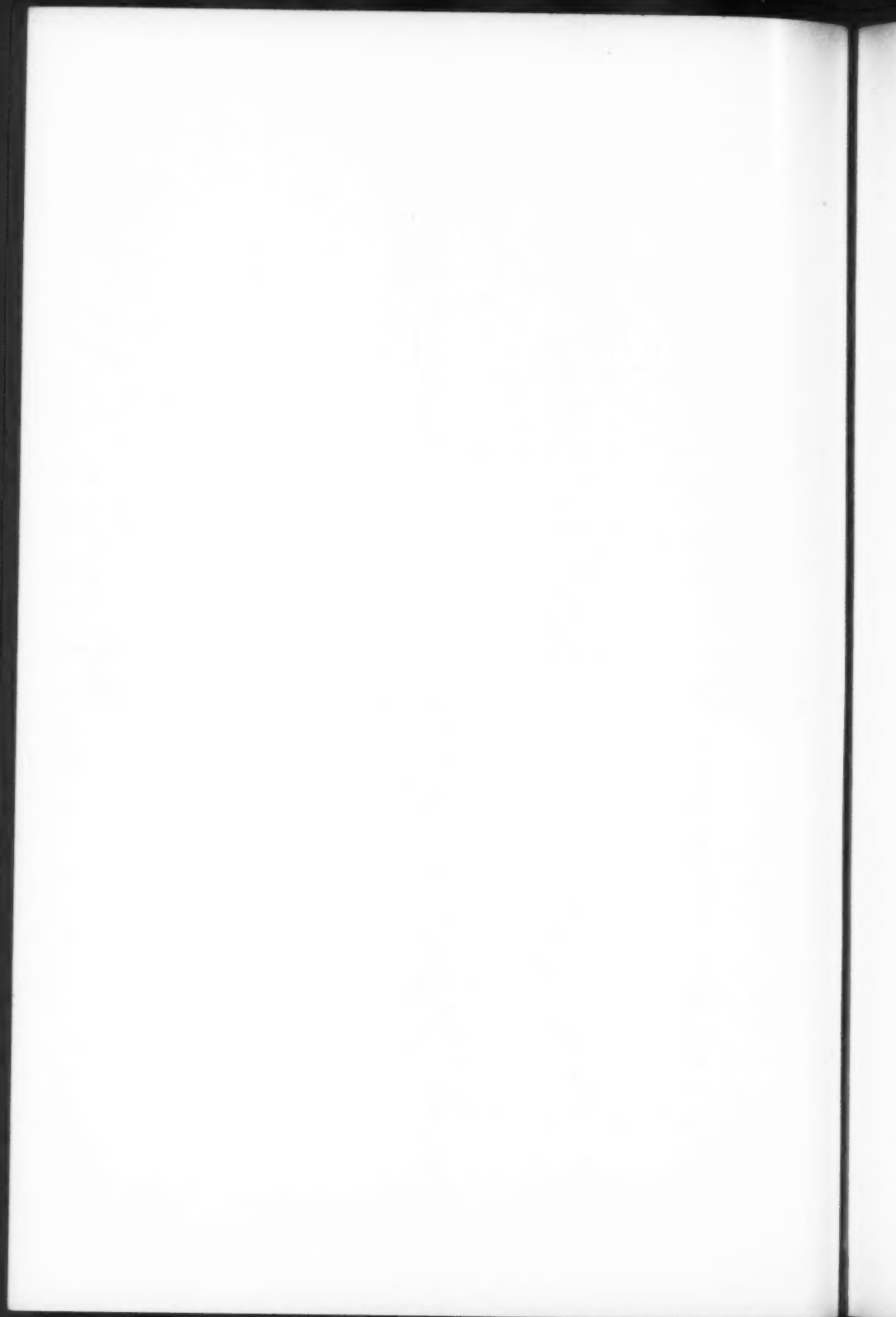
NON-OSTEOGENIC FIBROMA OF BONE *

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Not infrequently, one encounters in bone a lesion which we conceive as a benign marrow-connective-tissue tumor and which, because the basic tissue does not undergo osseous metaplasia in the course of the lesion's development, we are calling "non-osteogenic fibroma of bone." Its usual site is the shaft of some long bone (generally one of a lower limb) not far from an epiphyseal cartilage plate, and the lesion does not necessarily traverse the entire diameter of the bone in the affected area. Grossly, the lesion appears as a single focus or a group of smaller adjacent foci of yellowish or brownish fibrous tissue. Histologically, its essential pattern is composed of whorled bundles of spindle-shaped, connective-tissue cells loosely interspersed with small multinuclear giant cells, but in many places within a lesion collections of foam cells may be seen, along with other variations in histologic detail.

The lesion in question has previously been interpreted in a good many different ways; for instance, as "giant-cell variant of bone cyst or osteitis fibrosa," "healing variant of giant-cell tumor," "xanthic variant of giant-cell tumor," "solitary xanthoma or xanthogranuloma of bone" (usually implying a limited expression of Hand-Schüller-Christian's disease), and "fibrous osteomyelitis." It is the purpose of this presentation (founded on the observation of ten cases) to define and interpret "non-osteogenic fibroma of bone" as a clear-cut entity deserving recognition as such on the basis not only of its pathology but also of its clinical and roentgenographic manifestations. Although the term "fibroma of bone" does appear here and there in the literature, the case reports under that heading which we have studied have consistently been found to relate to a lesion having, as basic elements, both osseous and connective tissue, instead of to a connective-tissue lesion specifically *not* containing osseous elements. These fibro-osseous lesions most often represent the so-called "ossifying fibroma" or "fibrous osteoma" of bone, though some of them apparently represent solitary expressions of a dysplastic connective-tissue lesion of bone which we would call "fibrous dysplasia"—a lesion likewise not corresponding to "non-osteogenic fibroma."

CLINICAL ASPECTS

Age and Sex Incidence and Localization

In respect to age, our ten subjects ranged between 6 and 21 years, and all but two were between 8 and 16 years. As to sex, there was an even distribution of the cases. In regard to localization, in all ten the lesion was in a long tubular bone. Specifically, it was in a tibia in four patients, a fibula in four, a femur in one and an ulna in one, thus involving a long bone of a lower limb in all but one case. Within the particular bone affected, the lesion was always in the shaft, and almost always limited to the upper or lower third. However, there was always an inch or two, though seldom much more, of unaffected shaft between the lesion and the nearer epiphyseal cartilage plate (Figs. 1 to 6).

It is difficult to make a direct comparison, in respect to incidence and localization, between the findings in our cases of non-osteogenic fibroma of bone and the findings in apparently similar cases from the literature as reported under other names. This is so because the data supplied often do not permit one to sort out confidently all the pertinent cases from among others that are usually described along with them. Nevertheless, even against the obscure background of the general literature and the confusion of classification, the predilection of the lesion in the apparently relevant cases for older children and adolescents and for the shafts of long bones near but not at the epiphyses stands out. Though our records do not include any instances of non-osteogenic fibroma of bone in other than long tubular bones, there is reason to suspect that the lesion does occasionally appear also in other bones.

Clinical Findings

In most of our cases, the complaints were of only a few weeks' or months' duration before admission to the hospital, although this does not mean that the lesion itself was not of longer standing. It is probable that the lesion is one which progresses very inconspicuously and may lie dormant for some time before attention is drawn to it. About half of the patients reported their difficulty as beginning with some trauma of moderate severity to the general region in which the bone lesion was subsequently discovered. Thus, after a sprain, kick, or fall these patients suffered from pain and swelling of an ankle, knee, or wrist. In these cases, palpation revealed a point of bone tenderness and sometimes even of bone swelling, and when a roentgenogram was made the lesion was discovered. Most of the other patients, while giving

no history of trauma, likewise had pain and swelling of a joint, not of long standing, as their chief complaint. In these cases, too, it was only the roentgenogram that directed attention to the lesion in one of the bones entering into the formation of the joint which was the focus of complaint. In one of the cases in which the lesion was in the tibia, this lesion was discovered incidentally to roentgenographic examination of the femur for an osteogenic sarcoma. Altogether, there is nothing distinctive or characteristic about the clinical findings in cases of non-osteogenic fibroma of bone, so that one is dependent upon the roentgenogram for even a presumptive diagnosis.

Roentgenographic Findings

The usual location of the lesion in the upper or lower third of a long bone shaft at some distance from the nearer epiphyseal cartilage plate has already been emphasized. In this connection, it should also be noted that often the lesion does not extend across the entire diameter of the affected shaft area. It failed to do this in the four cases in which it was in a tibia and the one in which it was in a femur. It did do so in the four cases in which it was in a fibula and the one in which it was in an ulna. Thus, whether it does or does not extend all the way across the diameter of the bone depends upon the width of the shaft in the affected area, the lesion tending to be rather small and consistently being longer than it is wide. When it extended all the way across the shaft, the latter was found bulged out, uniformly or at least on one side. Even without reaching all the way across, it also occasionally bulged out the shaft in one place. Furthermore, when this was not the case, the lesion was still eccentric, that is, it hugged the cortex on one side (Figs. 1 to 6).

In the fibula and ulna (that is, in the cases in which the fibroma extended across the width of the shaft and expanded it somewhat) the lesion was usually about 3.8 cm. in length. It appeared as a more or less subdivided area of rarefaction, showing confluent or distinct locules. Some of the thin dividing "partitions" which it presented cast rather dense shadows. The cortex delimiting the area as a whole tended to be thinned and expanded over much of its scope, but still cast a clear-cut shadow. In a case showing a transverse infraction line, the cortex on the side of the infraction was found thickened by new bone apposition. Like the lesions extending across the width of the shaft, the eccentric lesions (those in the tibia and femur) were usually also not more than 3.8 cm. in length and less than this in width. When they did not expand the cortex upon which they abutted, this cortex was sometimes found sclerosed. Toward the medullary side, the lesion

was outlined by an encapsulating shell, usually rather thin but casting a dense shadow, and, as a rule, one or more "partitioning" shadows traversed the lesion irregularly.

Even from the roentgenogram alone one can often make the correct diagnosis. Certainly a small, eccentric, loculated lesion found abutting on the cortex of the shaft of a long bone, outlined by an encapsulating shell of bone on the medullary side, and not associated with notable thickening of the cortex, is most probably a non-osteogenic fibroma. So also (though a little less surely) is a small lesion located in, and expanding, the shaft of a fibula or ulna and appearing as a loculated area of rarefaction, although it should be borne in mind that such an area occasionally represents a solitary unicameral bone cyst. However, in any event, the definitive diagnosis must rest upon pathologic examination of the tissue occupying the affected shaft region.

Treatment

Non-osteogenic fibroma of bone is readily amenable to treatment. The cases discussed in this paper were all treated surgically, the procedure in most cases being thorough curettement of the lesion. In three cases in which the lesion was in the fibula, subperiosteal resection of the affected part of the bone was done. This seemed the easiest way of completely eradicating the focus of the disease in these particular cases, though, even in slender long bones, resection may not always be necessary. There were no recurrences. None of the patients received postoperative radiation therapy. Whether the lesion would be amenable to radiation therapy alone (that is, without surgical intervention) we cannot say. Of course, without the histologic examination of tissue made possible by such intervention it would be difficult to know whether the lesion being so treated actually represented a non-osteogenic fibroma of bone.

PATHOLOGY

The periosteum of the affected portion of the shaft is not particularly thickened, except at the site of an infraction undergoing repair. On exposure of the medullary activity, the lesion, as already noted, is likely to be found to be eccentric and abutting upon the cortex on one side only if the long bone affected is a thick one, or, on the other hand, extending across the entire diameter of the shaft if it is a slender one. The lesion usually consists of several more or less discrete but adjacent foci of tough tissue having a fibrous character (Figs. 7 and 8). The color of this tissue is brownish or yellowish, and, though some lesions may be more or less uniform in color throughout, others present a more mottled appearance, created by a mixture of yellowish and

brownish foci. As to the cortex of the shaft neighboring upon the brown-yellow tissue of the lesion, this may be found eroded and thinned in some places and abnormally thickened in others. Furthermore, each focus may be outlined in part by a thin shell of sclerotic bone, and some of the individual foci may also be separated from each other by bits of sclerotic spongiosa.

On microscopic examination, it appears that the general pattern of the stroma of the lesion consists of whorled bundles of connective-tissue cells (Figs. 9 and 10). However, the cellularity of the stroma varies from one lesion to another or from one focus to another within the same lesion. In accordance with the relative gross brownness or yellowness of the tissue, there is also variation in regard to the lesion's vascularity, although, on the whole, the latter is not great.

Thus, in a distinctly brownish lesion or focus, the stromal connective-tissue cells are spindle-shaped and closely compacted, being inter-spersed with but little collagenous intercellular material. Many of the stromal cells are likely to contain granules of hemosiderin in their cytoplasm, and it is mainly this that accounts for the brownish color of the lesion or focus as a whole, although some scattered capillary hemorrhages may also contribute to it. Irregularly dispersed among the stromal cells are small, often elongated multinuclear giant cells. These cells, sparse on the whole, may be more numerous and clustered together in some fields and especially about areas of recent capillary hemorrhage. The giant cells seem to be formed through fusion of the spindle-shaped stromal cells, and, like the latter, many of them contain granules of hemosiderin in their cytoplasm (Fig. 11).

In a distinctly yellowish lesion or focus, large and small nests of lipoid-containing foam cells are seen, admixed with and encircled by the stromal tissue (Fig. 12). The latter then consists of rather collagenous, spindle-shaped connective-tissue cells in winding thick strands or whorled bundles, honeycombed by the lipoid cells. It can be shown that the lipoid cells arise through conversion of the spindle cells into lipo-phages, and that the lipoids contained within the latter are, to a large extent, of the nature of cholesterol esters. On the whole, the more yel-low the lesion or focus, the more lipophages does it contain and the more collagenous does the intervening stromal tissue appear; and, furthermore, the less does it show of hemosiderin pigment in the stromal cells, or of multinuclear giant cells among them. Why the disappear-ance of the hemosiderin pigment and giant cells should parallel the appearance of foam cells in the lesion we do not know, but the fact that it does so is clear from the findings in areas representing intermediary stages of yellowness or brownness.

Thus, in an individual lesion, one may see fields in which the stromal

connective-tissue cells are rich in hemosiderin and interspersed with
giant cells, and other fields in which pigment-bearing cells and giant
cells are sparse or absent and foam cells are numerous. However, in
about half of our cases, the entire lesion failed to show any lipoid at
all, although the latter was sought for in frozen sections of material
stained for fat, and foam cells were looked for in paraffin sections pre-
pared from many areas of each lesion. Hence, as will be shown more
fully later on, it seems clearly unjustifiable to lay emphasis upon the in-
constant lipoid element by calling the condition a xanthoma or xantho-
fibroma of bone.

Furthermore, none of the lesions, of course, showed evidence of
osteogenesis as a feature of the cytology, and indeed the lack of bone
formation within these lesions is a consistent and striking finding. It is
true that individual foci may be walled off or delimited at their periph-
ery by a narrow zone of bone. Also, abutting upon the cortex of the
shaft, the lesion may even provoke the former to thicken in some places,
just as, in other places, it may erode it. However, in either case, such
bone formation represents a response of the neighboring tissue to the
lesion, and is not a feature of the lesion itself.

DISCUSSION

It is realized that calling the lesion under consideration "non-osteo-
genic fibroma of bone" raises questions of classification and nomencla-
ture. As a matter of fact, the Surgeon General's Catalogue and the
Index Medicus list hardly any references to "fibroma of bone" and
none to "non-osteogenic fibroma of bone." Furthermore, the various
textbook classifications of bone tumors include no such category as
"non-osteogenic fibroma." References to "ossifying fibroma," "osteo-
fibroma," or "fibrous osteoma" are considerably more common, but
these terms, indicating as they do that one of the inherent elements in
the lesions so classified is osseous tissue, distinguish these lesions from
the one we are considering here. How, then, is that lesion, which is by
no means a rare one, recorded in the literature? Most commonly, cases
representing non-osteogenic fibroma of bone are found described as in-
stances or variant forms of so-called localized osteitis fibrosa and as
instances or variant forms of giant cell tumor of bone.

There can be no doubt that some, though by no means all, of the
lesions which Geschickter and Copeland,[1] for instance, discuss as "giant
cell variants of the bone cyst in the metaphyseal ends of the long bones"
(p. 268) represent what we are calling "non-osteogenic fibroma of
bone." Also, their illustration (p. 300, Fig. 195) of the histology of
what they call "the giant cell variant of osteitis fibrosa" would do per-

fectly as an illustration of the lesion we are describing. Altogether, their terminology implies that the lesion in question is related both to solitary bone cyst and to giant cell tumor of bone, and that it actually represents something intermediary between them (p. 269). To follow their reasoning at all, one must bear in mind the opinion of these authors that solitary bone cyst (or osteitis fibrosa) and giant cell tumor are closely related lesions which have a common basis in an abnormal hyperplasia of osteoclasts at sites of endochondral ossification (pp. 289 and 308).

The reasoning of Geschickter and Copeland [1] which has just been outlined contains several fallacies. One is that bone cyst (osteitis fibrosa) and giant cell tumor are pathogenetically related lesions. Indeed, our own findings [2] indicate that solitary bone cyst starts apparently on the basis of a local disorder of development and growth of bone and certainly does not represent a focus of "osteitis fibrosa" which has become cystic. Furthermore, a solitary bone cyst usually has few if any tissue masses adherent to its wall, and such as may be present show clearly that they have their basis in the organization of hemorrhage. Again, our own findings in regard to giant cell tumor of bone [3] indicate that the basic cell of that lesion is not the giant cell but the stromal cell, and that this stromal cell is, of course, not an osteoclast but rather an immature marrow-connective-tissue cell. Finally, though in non-osteogenic fibroma the basic histologic pattern is that of intermingled stromal cells and giant cells, its resemblance to the giant cell tumor is only superficial. In the former as contrasted with the latter, the stromal cells are small and very spindly and show a strong tendency to collagenization and lipoid impregnation; the giant cells are small and sparse; and the lesion as a whole often provokes a perifocal osteosclerosis.

These very characteristics of the cytology of non-osteogenic fibroma have led other observers to regard this lesion as representing either a healing form [4] or a xanthic variant [5] of giant cell tumor. This idea is not valid either. In particular, non-osteogenic fibroma is observed most often in subjects below 20 years of age, while genuine giant cell tumor is rarely observed in subjects below this age. Furthermore, the fibroma nearly always begins in the shaft of a long bone, not far from an epiphyseal plate, but does not tend to extend into the epiphysis, while giant cell tumor usually begins in an epiphysis of a long bone though it tends to extend to the shaft. Again, non-osteogenic fibroma is usually a small lesion in comparison with a giant cell tumor. In addition, the scattered multinuclear giant cells which are often present among the stromal connective-tissue cells do not argue against designation of the lesion in question here as a fibroma, since medullary connective tissue,

even when rather mature, has a strong inherent tendency to form giant cells wherever it is proliferating. On the other hand, giant cells are likely to be absent in a non-osteogenic fibroma, or parts of it, where the lesion has undergone much lipoid transformation and collagenization, apparently in association with a diminution or regression of its growth activity. Finally, none of the giant cell tumors which we have studied showed substantial lipoid transformation or collagenization, or provoked a perilesional osteosclerosis such as would offer even slight justification for linking non-osteogenic fibroma with giant cell tumor or, specifically, for regarding the former as a healing or perhaps xanthic variant of the latter.

There can be no doubt that examples of non-osteogenic fibroma of bone have, in the past, been described as solitary xanthoma, xantho-fibroma, or xanthogranuloma of bone and such have usually been conceived in this connection as solitary expressions of lipoid granulomatosis or Hand-Schüller-Christian's disease. Pertinent cases have been described by Phélip,[6] Bahls [7] and Burman and Sinberg.[8]* This interpretation is likewise invalid, in our opinion. It is tempting because, in a non-osteogenic fibroma, many of the stromal cells may become converted into lipoid-containing foam cells. However, it should be borne in mind that in about half of our cases the entire fibroma failed to show any foam cells at all, although a careful and systematic search for them was made. Furthermore, even in those few lesions in which foam cells were abundant in some areas they were still absent in others, and in any case their presence was not associated with an inflammatory reaction. Altogether, when lipoid-containing fibromas of bone are compared histologically with genuine lesions of Hand-Schüller-Christian's disease, it becomes clear that there is no proper basis for regarding them as identical lesions.

In fact, we regard as very lax the current tendency to label lesions in bone as expressions of Hand-Schüller-Christian's disease merely because they contain some foam cells. For instance, Farber,[9] who observed foam cells in the lesions of eosinophilic granuloma of bone,[10] interpreted the latter condition as a variant of Hand-Schüller-Christian's disease. Other examples are provided by Snapper [11] and Landoff,[12] who, likewise observing a few foam cells in a lesion of fibrous dysplasia,[13] labelled that condition, too, as a variant of xanthomatosis or lipoid granulomatosis. If one used this line of reasoning, he would

* The article by Burman and Sinberg relates to one of the cases included in the present discussion and quotes one of us (H. L. J.) as having made an anatomic diagnosis of "lipoid granulomatosis (xanthoma) of bone" in that case. Aside from the interpretation given to the report we originally made in this case, we wish to record that we would not make that diagnosis now.

also have to say that certain cases of suppurative osteomyelitis, for instance, represent a variant of Hand-Schüller-Christian's disease, merely because in chronic stages they may reveal some foam cells in the inflammatory granulation tissue.

On attacking the question of nomenclature and classification from the opposite angle, it appears that though the term "fibroma of bone" has sometimes been used,[14] it seems not to have been applied to the lesion in question here. The term has most often been applied to lesions (particularly in the jaw bones) of the same character as those which have also been denoted as ossifying fibroma or fibrous osteoma. It has likewise been applied (again particularly in relation to the jaw) to lesions of the nature of solitary foci of fibrous dysplasia. In relation to other skeletal regions, lesions designated as fibroma of bone nearly always represent foci of fibrous dysplasia. Thus, for instance, the lesion described by Levinthal and Kirshbaum [15] as "fibroma of the metacarpal bone" is a clear-cut example, in respect to both gross and microscopic appearance, of fibrous dysplasia of the bone in question. As they stated, the expanded and otherwise modified metacarpal bone was substantially occupied by grayish white connective tissue showing some bony metaplasia and some giant cells, occasionally collected around bony trabeculae. The lesion which Mustakallio [16] referred to as "central bone fibroma" likewise represents fibrous dysplasia. This author stated that at the site of a central fibroma of bone the interior of the bone is filled, at least to a major extent, by a mass of connective tissue which is loose and reticular in some places and compact and collagenous in others. It is precisely this composition (usually with the addition of metaplastically formed bony trabeculae) that gives distinctive character to the lesion which we have denoted as fibrous dysplasia of bone—a lesion which affects only one bone or part of a bone in some cases and several or even many bones in others.

In choosing the term "non-osteogenic fibroma of bone" for the lesion under discussion we have meant to imply, as already noted, that the lesion represents a benign tumor, that it arises from the connective tissue of the marrow, and that in the course of the tumor's development the proliferating connective tissue does not undergo osseous metaplasia. We are aware that there are many who would question the propriety of calling the lesion a tumor at all. Indeed, some would argue that it represents merely an inflammatory condition and specifically something like a "fibrous osteomyelitis." However, its conception as an osteomyelitis (even in burnt-out form) is not made plausible by the addition of the modifying term "fibrous." This is merely an evasion, for there is certainly nothing in the histology of the lesion to indicate that it has, or

has ever had, a basis in inflammation. Nor is there anything in its histology to indicate that it may have a basis in reparative processes taking place after local trauma. On the other hand, a lesion which thus presents no vestiges of inflammation or reparative response, but which shows a cytologic pattern interpretable as having developed through the apparently unprovoked proliferation of autochthonous connective tissue must, on the basis of oncological usage, be classified as a tumor. However, it is a tumor of rather limited growth capacity. The absence from it of striking cellularity, mitotic division figures and nuclear atypism militates (like the clinical course) against calling it a fibrosarcoma, even of low grade, as is probably sometimes done.

SUMMARY

The lesion which is the subject of this paper is being called "non-osteogenic fibroma of bone" because we hold it to be a benign tumor formed from matured marrow connective tissue and not containing osseous trabeculae as an integral feature. In regard to the clinical findings, we noted that most of the subjects are older children or adolescents and pointed out the lack of characteristic clinical manifestations in connection with the disorder. We also noted that the usual site of the lesion is the shaft of a long tubular bone (most commonly of a lower limb), not far from the nearer epiphyseal cartilage plate. It was observed that the lesion tends to be a small one and may not traverse the entire diameter of the affected bone, especially if the latter is not slender. Accordingly, it was remarked that the lesion may show up roentgenographically as a sharply delimited, eccentric, somewhat loculated area of rarefaction, hugging and even bulging out the cortex on one side or, on the other hand, as a multilocular area of rarefaction traversing the bone and even bulging it out on both sides. As to its pathology, the lesion was described as consisting grossly of several discrete but contiguous yellow-brown fibrous foci whose basic microscopic pattern was found to be made up of whorled bundles of spindle-shaped connective-tissue cells loosely interspersed with small multinuclear giant cells, though, in some lesions, areas containing foam cells may also be present and even prominent. As to treatment, it was pointed out that thorough curettement or block resection of the affected area is all that is needed to abolish the disorder. Finally, we have tried to show why "non-osteogenic fibroma of bone" does not represent bone cyst (osteitis fibrosa) or giant cell tumor even in variant form, nor lipoid granulomatosis (Hand-Schüller-Christian's disease) in the form of a solitary lesion, nor a focus of "fibrous osteomyelitis."

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DESCRIPTION OF PLATES

PLATE 31

FIG. 1. Roentgenograph showing a lesion of small size, located in the tibia, about 4.5 cm. below the site where the upper epiphyseal cartilage plate had been, and hugging the cortex posteriorly. A delimiting shell of bone is seen, especially on the medullary side of the lesion. The patient was a young man, 21 years of age, in whom the lesion was asymptomatic and was discovered in connection with examination of the femur on the same side for an osteogenic sarcoma.

FIG. 2. Roentgenograph showing a lesion of about the same size and location as that shown in Figure 1. The cortex of the tibia toward the distal end of the lesion is somewhat thickened. The patient was a boy, 16 years old, who complained of pain in the knee of only 2 weeks' duration, starting after a football game.

FIG. 3. Roentgenograph showing a somewhat larger and longer lesion also abutting on the posterior wall of the cortex of a tibia. The upper limit of the lesion was 6.3 cm. below the upper plate of the tibia. The lesion appears multilocular with a delimiting shell of bone about it. The patient was a boy, 15 years of age, who went to the hospital because of pain and disability of the knee, of one month's standing, without antecedent trauma.

FIG. 4. Roentgenograph showing a lesion in the lower portion of the shaft of a fibula. The lower end of the lesion is about 2.5 cm. above the corresponding epiphyseal cartilage plate. The lesion appears multilocular with expansion of the diameter of the bone in a large part of the affected area. From the roentgenogram alone one might suspect, quite plausibly, that he was dealing with a solitary unicameral bone cyst, for instance, but actually the entire affected area was found filled by yellow-brown fibrous tissue (see Figs. 8 and 9). The patient was a girl, 10 years of age, who complained of pain of about 4 months' standing, in an ankle, and dated her difficulty from a kick in that area.

FIG. 5. Roentgenograph showing a lesion resembling that shown in Figure 4 but located in the lower portion of the shaft of an ulna. The patient was a boy, 8 years old, who complained of mild local pain, of 2 months' standing, said to have appeared first after a fall.

FIG. 6. Roentgenograph of a lesion in the upper portion of the shaft of a fibula. The upper end of the lesion is about 1.3 cm. below the corresponding epiphyseal cartilage plate. Periosteal new-bone apposition, representing a response to a transverse infraction, is seen on the surface of the cortex. (Compare with photograph of the specimen, as shown in Fig. 7). The patient was a girl, 7 years of age, whose history included pain, of about 2 years' standing, in the upper part of the left leg.
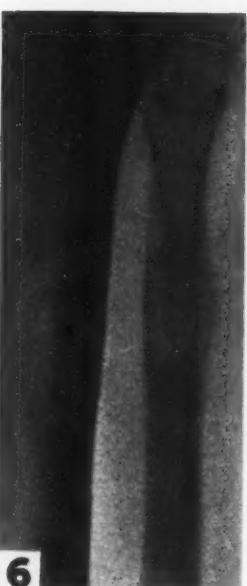
Jaffe and Lichtenstein

Non-Osteogenic Fibroma of Bone

PLATE 32

FIG. 7. Photograph of the resected portion (split longitudinally) of the affected fibula shown in Figure 6. Two adjacent foci of disease are seen which correspond precisely to the areas of rarefaction shown in the pertinent roentgenograph. For cytologic details, see Figures 10 and 11.

FIG. 8. Photomicrograph of a section prepared from the resected portion (split longitudinally) of the affected fibula shown in Figure 4. Although tissue is seen to fill the medullary cavity in the affected area, and the pertinent roentgenogram might suggest a cyst, there is actually no cavity in the region in question. The tissue of the non-osteogenic fibroma is very dark in the picture, not only because it was itself quite brown, but also because the section was rather thick. For some histologic details of the tissue composition in this lesion, as shown in a thinner section, see Figure 9. × 3.
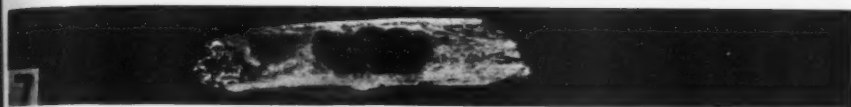
FIG. 9. Photomicrograph showing the general cytologic pattern of the lesion shown in Figure 8. A whorled arrangement of the stromal cells is seen, and scattered, larger dots which are the multinuclear giant cells. There is complete absence of osseous trabeculae within the connective-tissue focus. × 8.
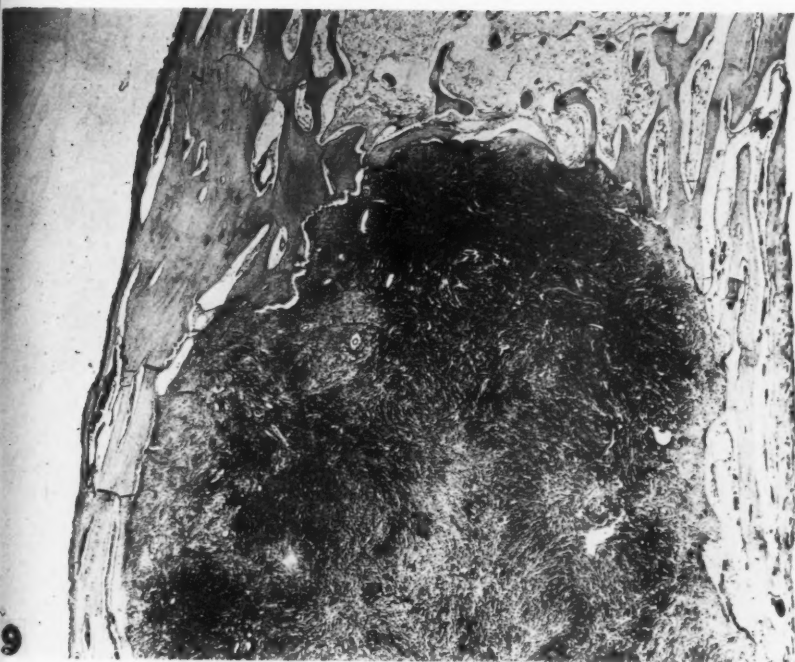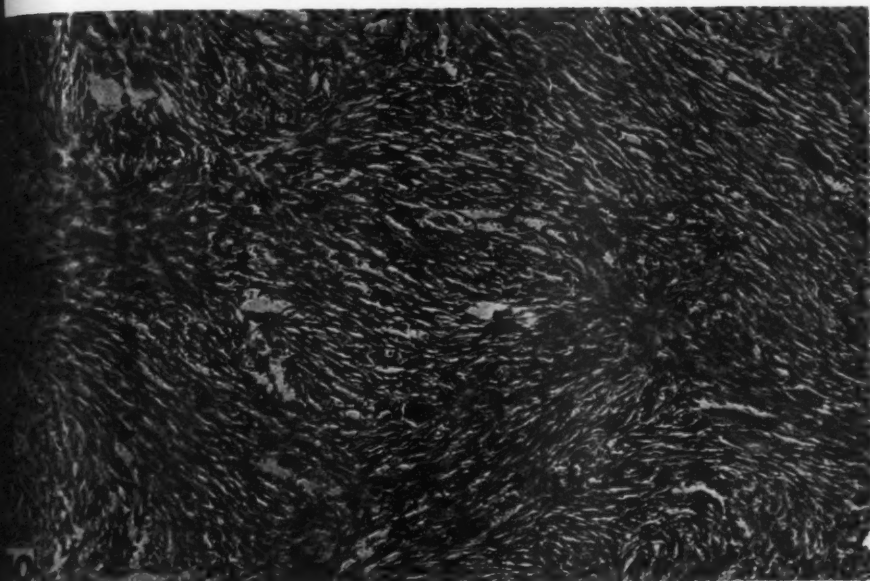
Jaffe and Lichtenstein

Non-Osteogenic Fibroma of Bone

PLATE 33

FIG. 10. Photomicrograph showing the cytologic pattern of the tissue foci in the medullary cavity of the resected portion of the fibula shown in Figure 7. A whorled arrangement of the closely compacted small spindle-shaped connective-tissue cells is seen, with scattered small multinuclear giant cells interspersed among them. Osseous trabeculae are absent. × 200.

FIG. 11. Photomicrograph showing in greater detail the pattern presented by Figure 10. Both the stromal connective-tissue cells and the giant cells are small. The difference in the stroma-giant cell pattern in non-osteogenic fibroma from that in a giant cell tumor under the same magnification can be seen by comparing this figure with Figure 1 of our article on giant-cell tumor.[3] × 450.

FIG. 12. Photomicrograph showing an area in a non-osteogenic fibroma in which some stromal cells are undergoing, or have undergone, conversion into foam cells. However, in half of the cases discussed in this paper no such foam cell areas were seen. × 200.

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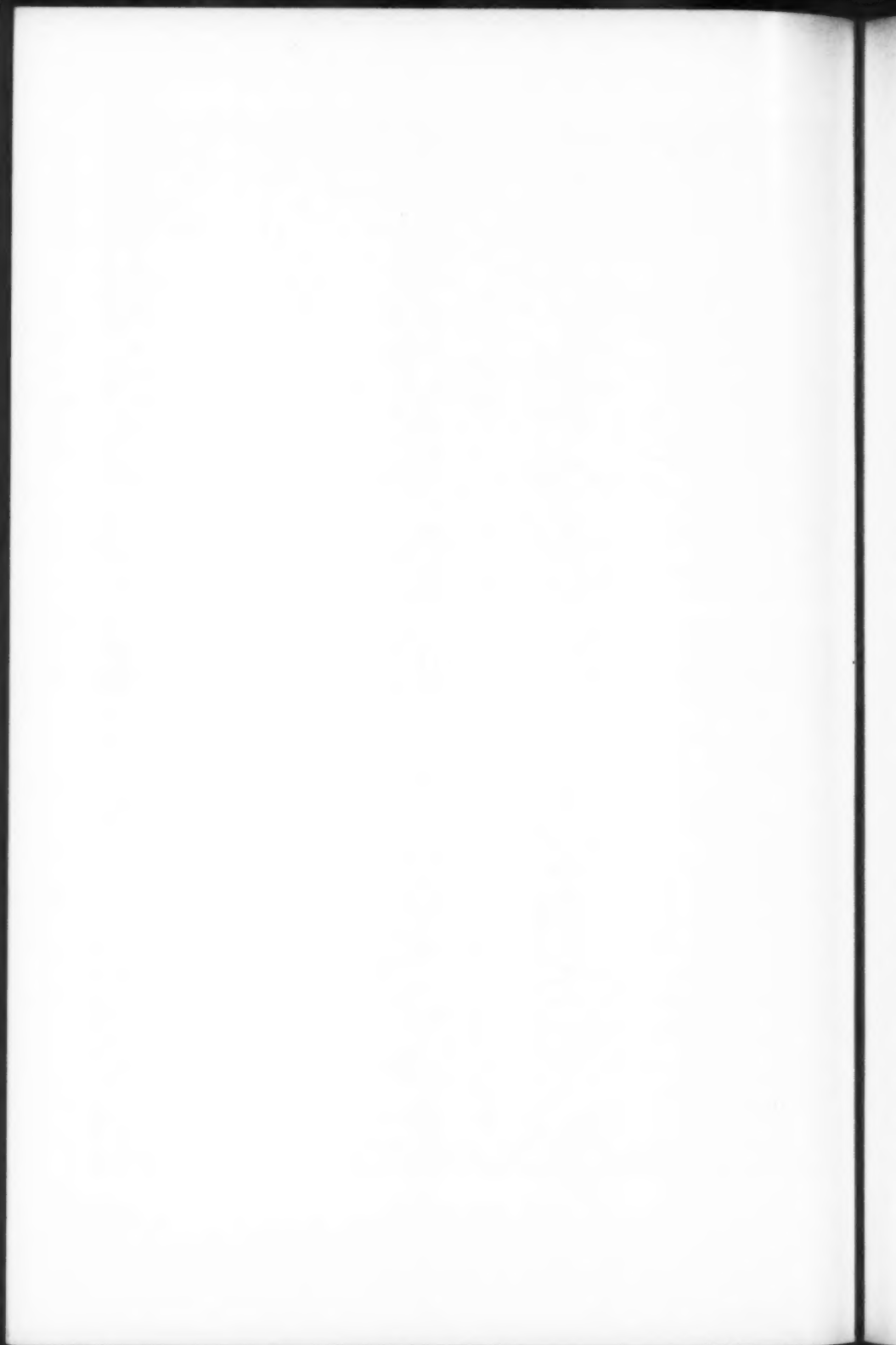
BRENNER TUMOR OF THE OVARY*

CASE REPORTS, DISCUSSION AND BIBLIOGRAPHY

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Hospital, Columbia University, New York, N. Y.)*

Fritz Brenner (1907) is given credit for the original description of
the tumor which bears his name, although he failed to recognize its true
nature, as did at least nine others before him. He called it "oophoroma
folliculare," and thought he was dealing with a form of granulosa cell
tumor. Von Mengershausen (1895), Orthmann (1899), Gottschalk
(1899) and Amann (1899) all described ovarian neoplasms which,
judging from their descriptions, were undoubtedly Brenner tumors.
Macnaughton-Jones reported a "solid ovarian tumor" in 1898, with
illustrations showing that it belongs in this group. Orthmann designated
his first two cases "adeno-fibroma cysticum carcinomatosum" and "fi-
broma papillare superficiale carcinomatosum," believing them to be
forms of ovarian carcinoma, but called his third case "adenofibroma
colloides ovarii." Schröder (1901), Lönnberg (1901), Gottschalk
(1902) and Voigt (1903) reported tumors of this type under a variety
of names. Voigt's case, and Orthmann's third case (which he con-
sidered to be benign) are of particular interest because the tumors
involved both ovaries.

Ingier (1907), in the same year as Brenner, also described two cases.
It is interesting that she called them "folliculoma ovarii," a term very
similar to the one Brenner had used, although they worked independ-
ently. Ingier's cases were both bilateral, but she failed to comment
further on this point.

It is noteworthy that four of the first seventeen tumors (23.5 per
cent) which appeared in the literature were bilateral, because it indi-
cated a much higher incidence of bilateral involvement than has been
generally realized. Dockerty and MacCarty (1939) reported ten
cases, none of which was bilateral. They stated that "95 per cent of
all Brenner tumors are unilateral." All fourteen of the cases reported
by Novak and Jones (1939) were unilateral. Proescher and Rosasco
(1936), in reviewing the literature, stated that they were able to find
only one bilateral occurrence, that of Weinzierl (1933). At the New
York Post-Graduate Hospital, two bilateral cases were recently en-
countered among our surgical specimens in a period of 10 days. A
review of our file since 1933 revealed two additional cases, one of which

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was bilateral. Therefore three of our four cases (75 per cent) involved both ovaries. In this review all of our fibromas and granulosa cell tumors of the ovary were restudied; the former because the fibrous tissue might easily obscure the epithelial elements, and the latter because Brenner classified his cases with granulosa cell tumors. It is impossible to determine how often bilateral tumors occur because, in more than 50 per cent of the reported cases the opposite ovary was either not mentioned or microscopic examinations were not done.

REPORTS OF CASES
Case 1 *

A white, married housewife, 42 years of age, was admitted to the New York Post-Graduate Hospital (F81419) in January, 1933, because of metrorrhagia and menorrhagia of 2 months' duration. Menses began at the age of 13 years and were "always regular." She had borne three children, and had had no miscarriages or operations. Physical examination revealed a firm, non-tender tumor of grapefruit size, in the right lower quadrant; the uterus was enlarged to the size of a 4 months' pregnancy, and displaced anteriorly and to the right. Supravaginal hysterectomy and bilateral salpingo-oophorectomy were performed.

On gross examination the uterus was found to be enlarged to 13 by 13 by 10 cm.; it was irregularly lobulated and there were several subserous, intramural and submucous fibromyomata. The fallopian tubes were grossly normal, but microscopically there was mild chronic inflammation. One ovary measured 3.5 by 2.5 by 1.2 cm. On section, numerous round, yellow areas up to 0.7 cm. in diameter were found in the stroma, as well as a thin-walled cyst containing clear fluid. The other ovary measured 3.8 by 2.0 by 1.8 cm., and on section there were round, yellow areas, up to 0.4 cm. in diameter, similar to those in the first ovary. In addition, there was an irregular, yellow, opaque area, situated beneath the surface, in the center of which was a minute cyst.

Microscopic examination† of the ovaries showed numerous corpora albicantia and sclerotic blood vessels. In a portion of each ovary there were localized areas of unusually dense stroma, in which numerous small, single and confluent islands of closely packed oval cells with oval granular nuclei were seen. In Dr. Meeker's original report, it was stated that "some of the nests were connected with strands of cells extending from the surface of the ovary," but this cannot be confirmed now because some of the original sections are no longer available. In one ovary the islands were composed of solid collections of cells, and in the other there were central cystlike spaces containing mucinous material and

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* This case was presented by Dr. L. H. Meeker to the New York Pathological Society on April 23, 1936, and published in the Proceedings of that organization for 1935–36.

† Sections were stained with hematoxylin and eosin, Mallory's phosphotungstic acid-hematoxylin, Mayer's mucicarmine and Foot's modification of Hortega's silver carbonate stains for reticulum.

desquamated cells. The cells around these "spaces" were columnar and arranged in radial fashion (Figs. 1 and 2). A few islands had poorly outlined borders, while others formed somewhat indefinite elongated strands of epithelium. In one ovary some of the epithelial nests were situated in the thin wall of the cyst described above. This cyst was lined by pseudo-stratified, flattened columnar epithelium (Fig. 3).

Case 2

A negress, 39 years of age, was admitted to the New York Post-Graduate Hospital (J32113) on September 20, 1940, complaining of pain in the right lower quadrant and back for 3 weeks. Menarche occurred at 14 years, and her menstrual periods were always "regular." She had three "miscarriages," but no children. Subtotal hysterectomy was performed in 1936 at another hospital, for fibromyomata uteri. In 1937 and 1938, operations were done for "intestinal obstruction." There had been no menses since the first operation. On examination pelvic masses were palpated on the right side, and interpreted as a cyst of the broad ligament and a tubo-ovarian abscess. Bilateral salpingo-oophorectomy and appendectomy were performed.

One ovary, 4 by 1.5 by 0.5 cm., was pale yellow, and contained a firm nodule, 1 cm. in diameter, as well as several small, blue cysts. On section, the cut surface of the nodule was gray with faint tan areas. The other ovary, 4 by 2 by 1.2 cm., also contained a firm nodule at one pole, 2 cm. in diameter. On section, the cut surface of the nodule was homogeneously gray and generally similar to the nodule in the first ovary. The appendix and oviducts were grossly normal.

Microscopically, the nodules were composed of abundant dense, interlacing strands of fibrous connective tissue, in which there were numerous large and small nests of epithelial cells. The nests were sharply circumscribed, and the cells closely packed together. The latter were round to oval with vesicular cytoplasm and relatively large, oval, pale-staining nuclei. The nuclei contained finely granular chromatin and small distinct nucleoli. No mitotic figures were seen.

Case 3

A white female, 45 years of age, was admitted to the New York Post-Graduate Hospital (J52388) on September 29, 1940. She had had an abdominal tumor since 1931, which was gradually increasing in size. Menarche occurred at 14 years; the menses were always regular, with no unusual symptoms. One pregnancy in 1933 ended in spontaneous abortion after 2 months. Physical examination revealed bilateral ovarian tumors. Both ovaries, the right fallopian tube and the appendix were removed.

On gross examination the right ovary formed a lobulated mass, 13 by 10 by 7 cm., with oviduct attached. The external surface of the ovary was smooth and glistening, with numerous small blood-vessel markings. On section, there were numerous lobules from 0.5 to 6.0 cm. in diam-

variety. Varangot, in 1937, collected 108, and the following year Novak
and Jones added 14 new cases, bringing the total to 122. I have been
able to find 166 cases, to which I add 4 more. Of these, 97, or 57 per
cent appeared in the last 10 years. The majority were unilateral; only
10 were bilateral, including a questionable case of Frankl's (1927).
The 3 cases reported here bring the total number of bilateral cases
to 13.

CLINICAL ASPECTS

In Table I the cases are classified according to age: 79, or 46.5 per
cent were 51 years of age or over, and 41, or 24.1 per cent were between
41 and 50 years. In respect to location, 38.9 per cent of the tumors
occurred on the right side and 33.5 per cent on the left; 7.6 per cent
were bilateral, and in 19.2 per cent the side was not stated. The young-
est patient was 20 years and the oldest 77 years of age.

The growth of a Brenner tumor is not attended by any recognizable
group of symptoms. Uterine hemorrhage occurred in less than one-
fourth of the cases. A "tumor in the abdomen" was the most common
complaint. A considerable number were found accidentally, either at
operation or at necropsy. Unlike the granulosa cell tumor, the Brenner

TABLE I

Classification of Patients with Brenner Tumors According to Age and Site of Tumor

Age in years	Right ovary only	Left ovary only	Bilateral	Side not stated	Total
0–10	0	0	0	0	0
11–20	1	0	0	0	1
21–30	5	3	2	1	11
31–40	14	3	2	7	26
41–50	14	18	3	6	41
51–60	17	20	4*	6	47*
61–70	11	12	1	3	27
71–80	5	0	0	0	5
Not stated	0	1	1	10	12
Total	67	57	13*	33	170*

* One questionable case.

tumor secretes no hormone and consequently produces no alterations
in sex characteristics.

MORPHOLOGY

Brenner tumors may vary in size from very minute nodules to as
large as a man's head. Giles reported a case in 1909 as "adenocarci-
noma?" in the wall of a multilocular cyst, which weighed 18 pounds;
Davison and Neiman (1934) described a Brenner tumor of the solid
type which weighed 15 pounds. The neoplasm is slow-growing and only
rarely reaches large proportions. Grossly, the solid variety, which con-

stitutes about 70 per cent of all Brenner tumors, resembles a fibroma. On section, there is frequently a yellow tint to the cut surface. Gaines (1936) stated that "under magnification with the hand lens, small cavities may occasionally be discerned, varying from pinhead to cherry size, and containing an opaque, viscid, yellow brown fluid." The second type consists of a solid tumor in the wall of a cyst, usually a pseudomucinous cystadenoma. These tumors fall into Meyer's classification under groups (a) and (b), respectively.

Microscopically, the solid tumors are composed of abundant fibrillary connective tissue in which small nests of compact, polyhedral epithelial cells are embedded. The cells frequently become stratified and bear a strong resemblance to squamous cells. They may be cuboidal or cylindrical, but very rarely ciliated. Mitotic figures are not observed. Novak emphasized the presence of the characteristic nests of epithelial cells and the fibromatous connective tissue groundwork in combination and stated that both must be present to justify the diagnosis of Brenner tumor.

In most tumors there is some tendency, however slight, to central cystic degeneration of the epithelial nests. The superficial layer of the cystic portion frequently assumes a columnar character with mucoid secretion similar to that which occurs in pseudomucinous cystadenomata. It is this feature which helps differentiate a Brenner tumor from a granulosa cell neoplasm. On the other hand, granulosa cell tumors contain lipoid material which is entirely absent in the Brenner neoplasm. In addition, Brenner tumors contain glycogen (Meyer, and von Szathmáry) which is not found in granulosa cell tumors.

With the Foot silver stain the fibrillary character of the stroma is clearly demonstrated. The whorls and strands of connective tissue extend up to, but do not penetrate, the basement membrane surrounding the epithelial nests. The cells within the limits of the basement membrane are completely devoid of silver granules.

Novak and Jones agreed with Meyer's view that "at least a certain though small proportion of pseudomucinous cysts have their origin in Brenner tumors." Meyer also stated that some serous cystadenomas may have a similar origin, though no cases of this type have been observed.

Brenner tumors are benign lesions and no recurrence or metastases have been reported. A case reported by Tavildaroff was said to have recurred in the other ovary after a few months (Mandelstamm, 1932). Undoubtedly this was not a recurrence but merely another example of a bilateral growth, which either was not recognized at the initial operation or grew after the first ovary was removed.

SUMMARY AND CONCLUSIONS

Four cases of Brenner tumor of the ovary are reported, three of which were bilateral. A review of the literature revealed 166 cases previously reported under a variety of names. Only ten of these cases (6.0 per cent) were bilateral, including one doubtful case of Frankl's. Bilateral occurrences do not indicate metastasis. The tumor constitutes a distinct entity and is not to be confused with granulosa cell tumors.

The histogenesis of the Brenner tumor is not known, although there seems to be some relationship to Walthard rests. The use of the name "Brenner tumor" is recommended until it can be classified on a histogenetic basis.

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DESCRIPTION OF PLATE

PLATE 34

FIG. 1. Case 1. Brenner tumor of ovary showing solid nests of cells in dense fibrous tissue stroma. × 82.

FIG. 2. Case 1. Cystlike spaces in nests of cells. The inner cells exhibit a radial arrangement and are columnar. × 82.

FIG. 3. Case 1. Nests of tumor cells in the wall of a large cyst. × 82.

FIG. 4. Case 3. Brenner tumor with superficial resemblance to carcinoid tumors. × 82.
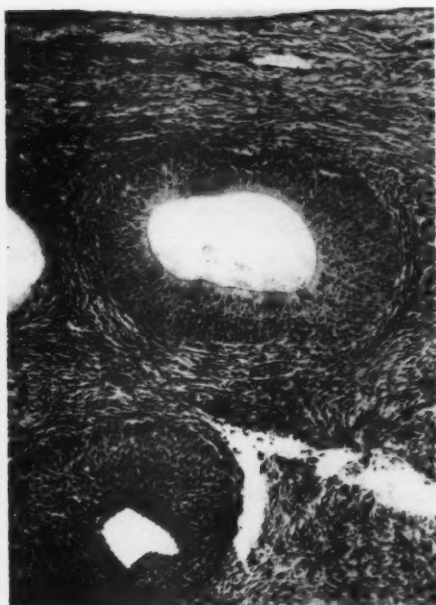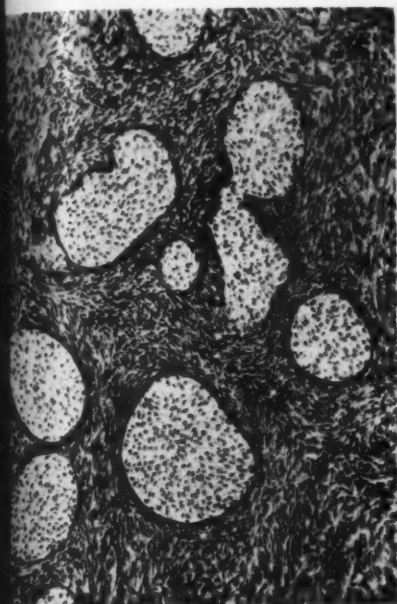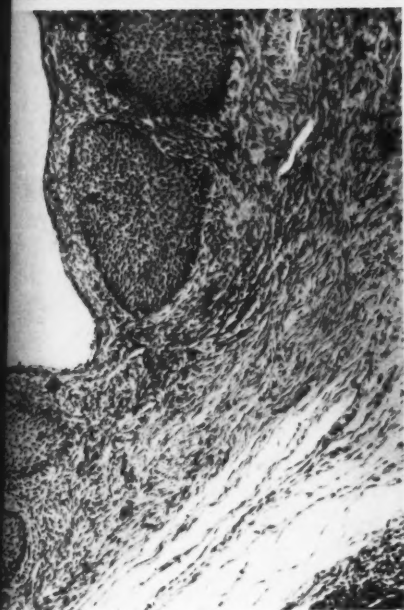
PLATE 34

2

4

Brenner Tumor of the Ovary

STUDIES ON AMEBOID MOTION OF MOTOR NERVE PLATES *

II. Pathologic Effects of CO_2 and Electricity on the Explosive Ameboid Motion in Motor Nerve Plates in Intercostal Muscle

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The explosive structural changes produced in motor nerve plates and related muscle striae by either strong carbon dioxide or electrical stimulation are unknown. Evidence has been presented which casts serious doubt on the prevalent opinion that the nerve plate in a mature and specific striped muscle fiber remains constant in size, shape and internal structure during the alternate phases of contraction and relaxation, and in contracture or rigor.[1]

Evidence is likewise presented in this paper which casts serious doubt on the prevalent concept that muscle fibers are permanently fixed into two types; namely, coarsely and finely striated. The manifold forms of the motor nerve plates and structural variations of the related muscle cross striations defy any simplification by a static morphologic classification. An understanding of the dynamics of functional ameboidism of the motor nerve ends and the influence of the changeable capillary chemistry in producing the different periodic structural changes in striped muscle may help to clarify this complex problem. At any event, only photographic evidence that others may evaluate of structural changes in muscle and nerve is of importance in the present state of confusion. Free-hand drawings and verbal descriptions have not clarified the problem.

Some of the recent excellent reviews that demonstrate the complexity of the problem which exists in reference to the normal structure of either motor nerve ends or muscle have been made by Cobb,[2] Needham,[3] Hines,[4] Jordan,[5] Hinsey[6] and Zeit.[7]

It is firmly established that protoplasmic movement, or ameboidism, occurs during the initial growth and during the regeneration of nerves. Ameboid movement at synapses during functional activity of the nervous system was assumed by Cajal.[8] He discovered that ameboidism was a reality by the gradual changes of position of growth cones of both embryonic and regenerating nerves. This movement was estab-

lished by the study of progressive changes in position of growing nerve terminals in fixed material. Observations of the actual protoplasmic movement in the growth of living embryonic nerves in tissue culture were made first by Harrison,[9] then by Lewis,[10] and were confirmed by numerous other workers, especially recently by Speidel.[11]

Evidence was presented for a rhythmic alternation of the dilatation and retraction of the motor nerve plates during the mature functional activity of intercostal muscle.[1] In the fractional contraction of muscle the resting or relatively relaxed fibers were characterized by retracted plates associated with coarse, widely spaced cross striations. The strongly contracted intercostal muscle fibers during hyperpnea by carbon dioxide had dilated nerve plates and fine, closely spaced cross striations. There were many transitional stages between these two extremes. The surface area of the motor nerve plate was increased during contraction of the muscle fiber. By physiologic methods Cooper [12] studied the relation of active to inactive fibers in the fractional contraction of muscle.

Evidence has been presented that the dark, condensed nodes and the light, rarefied internodes of cross striations in fixed striped muscle in heat rigor are the two structural expressions of a "frozen-standing" system of longitudinal compressional waves. These mechanical pressure waves accompany the strong capillary chemical changes within the muscle, and are expressed as periodic bands when the working muscle substance is confined and enclosed in an elongated, microcapillary space of relatively constant volume. The variable number of these cross bands is directly related to the variable speeds, concentration and composition of the reversible or irreversible capillary chemical reactions. When the speed of capillary chemical change, influenced by temperature, is at an optimum high, there is a greater number of cross striations in the same muscle fiber than when the speed is low. The explosive compressional waves that accompany the capillary chemical changes in the normal microcapillary muscle fiber determine the structural arrangement of the protoplasm, in space, into zones of condensation and rarefaction.[13]

Buchthal [14] stated that "The activity of a muscle in every respect is an interference phenomenon, and it is only possible to make a real analysis of the mechanical and electrical response of the muscle, and also of its optical properties in relation to its structure, if one works with single muscle fibres. The more we know about the minute structure of the fibre, the more we shall know about the mechanism of a muscle's ability to vary its length and tension."

Presumptive evidence that the motor nerve plates physically vibrate

was presented on the histologic basis of the radiation patterns of muscle striae related to the nerve ends.[15] More direct evidence of the physical vibration, or alternation of large and small size of the same nerve plate, was found in intercostal muscle from rats, the respiratory rate of which was accelerated by carbon dioxide.[1] The reappearance of the granular sole plate of Kühne surrounding the retracted axonal ramifications during relaxation and the disappearance of this granular material in the expanded axonal ramifications during contraction lends support to the chemical theory of impulse transmission. The periodic appearance and disappearance of the granular sole plate of Kühne lends support to the theory that there is a substantial transmission, rather than that of a more subtle electric current, from the motor nerve end to the muscle. This experimental histologic evidence, therefore, lends support to the chemical theory of conduction in nerve to muscle at the myoneural junction.

These structural changes during function also give support to the very recent theory of Rosenblueth [16] that the nerve plate in muscle is a chemical secretory microgland. This morphologic evidence of the explosive periodic change in the structure of the nerve plate and muscle in functional activity likewise favors the recent "trigger concept" of the rhythmic explosions in muscle proposed by Fenn,[17] as follows: "Thus at the desire of the experimenter a whole series of new chemical reactions can be thrown into activity in an orderly and reproducible manner. This sudden transformation so produced may be properly described as an explosion. The muscle is a self-cocking explosive machine with a convenient trigger. The explosion turns chemical potential energy into mechanical energy . . . The forces developed into a muscle 'explosion' are of amazing magnitude." Sacks [18] concluded his excellent review on the chemistry of muscular contraction by this final pertinent statement: "Finally, and most elusive of all, there remains the question of the mechanism by which the muscle transforms the chemical energy derived from oxidative or anaerobic reactions, into the mechanical work which is its function in the body."

Hines [19] stated that the nerve ends in striped muscle are "morphologically lawless." Wilkinson's [20] developmental theory was proposed to explain the morphologic variations in motor nerve plates in striped muscle. He wrote:

"These series of figures seem to indicate the probability that at least some of the *terminaisons en grappes* are immature forms of somatic motor plates and, morever, illustrate the various stages in the development.

"It is necessary to point out, however, that although the grape-like endings are here regarded as immature motor endings, it is not implied that they will necessarily eventually develope during the life of the animal into plate-like endings. Whether

they are endings which represent a reserve and can under certain conditions develope into plate-like endings or are endings in which development is definitely arrested, cannot be stated with any certainty. All that can be said, is that these grape-like endings can be arranged in a series which shows an increasing degree of resemblance to plate-like endings, and for that reason are here called immature motor endings. The condition of affairs, however, found in mammalian embryonic material and in young mammals and also in material from nerve regeneration experiments, seems to lend support to this conception."

Since the periodic changes in the size, shape and internal structure which occur during the normal functional activity of a specific motor nerve plate are unknown, it is not surprising that the sudden and explosive pathologic structural changes in nerve and muscle produced by strong stimulation with carbon dioxide and electricity are likewise unknown. Relatively chronic changes in motor nerve plates produced by starvation (Denny-Brown [21]), dietary deficiency in beriberi (Woollard [22]), and nerve degeneration (Hines,[19] Cajal,[23] Tello [24] and numerous others) are well known.

It is appreciated that for absolute conclusiveness, there is one weak link in the chain of evidence for the periodic motion of motor nerve plates, and this is the lack of an actual cinematographic record of the rhythmic expansion and retraction of one motor nerve plate during the functional activity of its muscle fiber. It is hoped that the technical difficulties involved in this problem will soon be solved. However, Cajal,[8] 50 years ago, came to the conclusion that ameboid motion lay at the basis of the growth of nerves, and produced evidence derived from fixed tissues before the motion picture was used in science. Very little imagination is needed to visualize the periodic changes in the motor nerve plates in relation to the cross striations of the muscle fiber when adequate photographic evidence is available.

I am familiar with no photographic evidence in the literature wherein the morphology of the nerve plates and of related muscle striae are both presented in continuity under normal and abnormal experimentally controlled conditions in the same animal. Kühne's [25] exhaustive comparative study and excellent paper with 346 drawings presented the structural differences in the nerve plates in different species of animals, but it ignored the related muscle structure. He neither presented evidence, nor reached the conclusion, that functional ameboidism was the underlying factor of the pleomorphism of the nerve plates and of their effect on the muscle striae in one muscle.

The purpose of this paper, therefore, is to present conclusive photomicrographic evidence in the form of a permanent atlas of the normal and abnormal pleomorphism of motor nerve plates due to functional ameboid motion. The immediate explosive changes in the size, shape

and internal structure of motor nerve plates in the right intercostal muscle of the same animal (white rats nos. 25 and 57) are produced as explosive effects of carbon dioxide and electrical stimulation. Fortunately the apparently abundant photomicrographic evidence with legends is clear enough, without a long detailed morphologic description; and this objective evidence is needed at the present time in order that others may evaluate it and make their own interpretations. The exact and clear presentation of easily verified facts necessitates the large number of illustrations in view of the contemporary state of confusion regarding the significance of the structural variations of the motor nerve plate and the related muscle cross striations in the physiologic and pathologic motions of muscle with its innervation intact.

MATERIALS AND METHODS

Rats were anesthetized by the intraperitoneal injection of nembutal, 25 mg. per Kg. The hair was clipped with the electric clipper over the right dorsal aspect of the thorax. A 1 inch vertical incision, 1 cm. to the right of the midline of the spine, was made through the skin and muscle down to the ribs. The sixth, seventh and eighth ribs and related intercostal muscles were quickly excised in one piece beginning medially 1 cm. from the spine and extending 1.5 cm. laterad.

This excised part of the thoracic wall was run through the gold chloride technic and served as a control of the relatively normal motor end-plates in the intercostal muscles of the right side. The bleeding was quickly controlled by hemostats and ligatures. The skin was sutured over the thoracic gap and the animals immediately placed for 1 to 10 minutes in a jar, the air of which contained roughly 15 to 30 per cent of carbon dioxide. The rats were removed from the jar and a second quick excision of the right second, third, fourth and fifth ribs and attached intercostal muscles of the right thoracic wall was made. This excised portion was at once run through the gold chloride technic for microscopic study of the teased muscle fibers and motor end-plates. After again suturing the skin, animals numbered 1 to 25 were allowed to breathe normal air for a period of $3\frac{1}{2}$ hours, and then were decapitated. In another group of rats (nos. 26 to 75) the same procedure was carried out except that the animals were killed immediately by electrocution instead of decapitation. This was done immediately after the second excision of the right second, third, fourth and fifth ribs and attached intercostal muscles of the right thoracic wall. The rats were suddenly killed by electric stimulation for 1 minute, through the spinal cord, with one electrode in the cervical region and the other in the lumbar region. The ordinary alternating current of 120 volts, 1 ampere,

was used. The ninth, tenth and eleventh right intercostal muscles were then excised, subjected to the gold chloride technic and teased.

The motor end-plates in the intercostal muscle, fixed after different rates of respiration, in the white rat (*Mus norvegicus*), were stained by the Bielschowsky method of silver impregnation as modified by Boeke;[26] by the *intra vitam* methylene blue method of Ehrlich as modified by Huber [27] and by Huber and DeWitt;[28] and by the Ranvier gold chloride method as modified by Wilkinson [29] and by me. The continuity of the medullated nerves, motor nerve plates and related muscle structure is best studied in teased muscle fibers after the following gold chloride method:

The muscles are cut with a sharp surgical scissors into pieces about 3 to 5 mm. thick and fixed in filtered, fresh, full-strength lemon juice. After 10 to 15 minutes, the lemon juice is decanted and a 1 per cent solution of gold chloride poured over the tissue without any washing in distilled water. (It was found that better results were obtained when the gold chloride solution was made up a day or two before use.) The bottle containing the metallic salt and tissue is kept in the dark until the bits of muscle assume a uniformly golden appearance. The time during which this is accomplished will vary from 10 minutes to 1 hour, so the only criterion that can be depended on for sufficient impregnation is the color. If left in the gold chloride solution until brown, the technic will prove to be unsuccessful. Without washing in distilled water the tissue is transferred to a mixture of one part of formic acid to three parts of distilled water and placed in the dark for 12 hours. (In the usual methods described, the muscle tissue remains in the acid solution from 24 to 48 hours. However, it was found by experience that when the tissue was cut thinly and uniformly so that just enough of the metallic solution penetrated the tissue evenly, the gold was nicely reduced and the muscle tissue was beautifully stained a reddish blue color with the nervous elements black, within 8 to 12 hours. If a longer period was required, it indicated a fault in technic in using the gold chloride solution, so that results were not good in any such case.)

After 12 hours in the formic acid solution, the pieces of muscle tissue are washed once in tap water and quickly placed in a mixture of equal parts of 50 per cent alcohol and chemically pure glycerin. This prevents the swelling that has been so often reported as occurring when the tissue was placed in pure glycerin.

A bit of tissue is removed, oriented in a drop of glycerin on a 1 by 3 inch slide with a pair of sharp teasing needles, and gently spread with a coverslip, care being taken not to do too much teasing with the needles so as not to disrupt the integrity of the muscle fibers with respect to each other and to their nerve endings. The slips are sealed to the slide with a ring of clarite, a nitrocellulose product far superior to the old paraffin. Many of my slides so mounted have kept for over 2 years without any noticeable deterioration. The difficulty with the old method has thus been obviated; namely, leakage through the paraffin ring allowing entrance to saprophytic organisms which produced decomposition beneath the slip.

Seventy-five rats were used in this study and over 5000 slides of teased muscle were made during the past 6 years. The motor nerve endings observed during this period would approach one million.

The Bielschowsky silver technic reveals the fine neurofibrillar, reticular nature of the fronds of the axon. The sarcoplasm of the sole plate

is clear and unstained by methylene blue. The gold chloride method, however, demonstrates the sole plate, when present, to be composed of fine granules. There is a narrow, clear area between the fronds or branches of the terminals and the granular muscle sole plate. In some expanded plates there is direct continuity between the serrated projections of the terminations of the axon and the dark muscle striae. The ovoid nuclei of the sole plate remain unstained by the gold method and form clear spaces surrounded by dark granules. The periterminal network of Boeke [26] is best seen in expanded nerve plates after staining by the silver method. It forms a foamlike reticulum between that of the nerve terminals in the plate and the periterminal network in the sarcoplasm of the sole plate. In the expanded nerve plates, which appear to be a chemical secretory apparatus, there is an apparent finer structural continuity between the terminal nerve plate and the surrounding muscle sole plate when stained by the silver method. In the retracted plates this apparent continuity is lost. The line of demarcation between nerve terminals and sarcoplasm is not a constant one. It depends upon the state of functional activity in which life is stopped and the tissue fixed. Although the structural integrity of both muscle and nerve is preserved at the myoneural junction, it appears that some chemical secretory granules pass from the nerve end to the muscle in some rhythmic manner.

The magnitudes of the muscle fibers are slightly enlarged by gentle compression and by the technic of preparation. The coarseness and fineness of the cross striations in the muscle fibers are in about the same relative proportions as during life in spite of the histologic technic. The continuous compression by the coverslip is counteracted by small supporting bits of coverglass. In photographing teased preparations of muscle fibers from 30 to 150 μ thick it is obviously impossible to bring simultaneously into focus all of the structures seen in any particular field. This difficulty, however, was partially surmounted in some photographs by making multiple exposures at different focuses on the same photographic plate. Careful observations were made to obtain records of those motor end-plates in which the cross striations were in the same focus as the related nerve plate. In variations of the focus there were changes in the pattern of muscle structures, especially in the thick fibers.

Experimental and Histologic Results

The respiratory rate after nembutal anesthesia in the white rats varied from 25 to 55 per minute. During excision of the piece from the right thoracic wall the rate increased to 60 to 80 per minute. This was

the reason for making the first incision through the ribs along the dorsal aspect, thereby severing the nerve supply to the excised muscles quickly and before the respiratory rate increased. These muscles were used as relatively normal controls. The rats were then placed in carbon dioxide for 1 to 10 minutes. The respiratory rate immediately was raised and varied from 130 to 200 per minute. When the rate of labored breathing fell below 100 per minute, the rats were taken out of the jar. The second excision of the right second, third, fourth and fifth ribs and attached intercostal muscles was quickly made. The respiratory rate fell to 60 per minute within 30 minutes while the rats breathed normal air, and remained at this rate until the animals were decapitated $3\frac{1}{2}$ hours later. By this simple means the histologic changes in the same group of muscle motor nerve plates in the same animal (rats nos. 1 to 25) were observed under different degrees of intensity of functional activity stimulated by the chemical method of carbon dioxide.

In another group of rats (nos. 26 to 75) the above procedure was repeated except that the animals were killed by electrocution instead of by decapitation. This was done immediately after the second excision of the right third, fourth and fifth ribs. In this series of animals the effect of respiratory superactivity by carbon dioxide and the lethal effect of electricity were superimposed.

The motor nerve plates from the right intercostal muscles in the relatively normal controls, excised prior to carbon dioxide stimulation, varied from the retracted state with wide, short fronds 21 to 25 μ in diameter (on middle muscle fiber Fig. 2, on upper muscle fibers Figs. 3 and 4, and middle muscle fiber Fig. 7) to the expanded state with long, attenuated pseudopodia 52 to 55 μ in diameter (on lower muscle fibers Figs. 3 and 5). These were the extremes in the dimensions of 2000 normal nerve plates.

The normal and abnormal retracted motor end-plates with wide, coarse fronds are related to coarse, widely spaced cross striations (Figs. 1, 2, 3, 4, 7, 9, 11, 14, 22, 25, 32, 36, 40, 51 and 59). The expanded plates with narrow, elongated processes are related to fine, closely spaced cross striations (Figs. 1, 2, 3, 4, 5, 6, 8, 13, 15, 30, 31, 34, 35, 39, 50, 58, 63 and 64). This relationship of the retracted and expanded motor nerve plates to a different number of cross striations is clearly demonstrated in Figures 25 and 31.

There are 11 dark cross striations related to the retracted nerve plate in the relaxed muscle fiber (Fig. 25). There are 32 dark cross striations related to the expanded motor nerve plate in the active muscle fiber (Fig. 31). This differential numerical relationship of the muscle striae related to the retracted and expanded nerve plates is also related

to physiologic and pathologic functions at the myoneural junction. This finding confirms the classification of muscle fibers by Hines [19] into coarsely and finely striated. There was, however, a closely graded series of transitional stages in the structure of the muscle fibers between these two extremes with variations in the number of the cross striations in the muscle. This agrees with the observations reviewed by Cobb,[2] Needham,[3] Hines,[19] and Hinsey.[6]

The prepared fibers from the right intercostal muscle stimulated by carbon dioxide to superactivity had, in 2000 measurements, 673 plates that varied from 29 to 55 μ in diameter. The largest motor nerve plate expanded by carbon dioxide stimulation of the respiratory center reached the diameter of 112 μ (Fig. 50). In 2000 measurements of the diameters of the stimulated motor nerve plates, 1327 varied from 55 to 91.4 μ and had 2 to 7 elongated, attenuated, distinct processes with periodic enlargements. These thin processes were separated by wide meshes and were related to fine, closely spaced cross striations. In some places the protoplasmic projections of the nerve plates formed anastomoses, and on some muscle fibers there were two discrete motor nerve plates. These were the two terminals of the two medullated branches of a single axon.

The sole plate of Kühne formed a dark, granular rim around the retracted and slightly expanded axon of the normal motor plates (Figs. 4, upper muscle fiber; 7, middle muscle fiber; 9, upper muscle fiber; 11 and 14, upper muscle fibers; 25, 26, 27, 32, 36, 51 and 59). This granular sole plate of Kühne disappeared in the greatly expanded end-plates related to fine, closely spaced cross striations (Figs. 3, lower muscle fiber; 8, 13, 30 and 31). There was a reduction by 80 per cent in rats nos. 1 to 25 in the number of motor nerve plates expanded beyond 55 μ in diameter in the right ninth, tenth and eleventh intercostal muscles after the animals were again breathing normal air for $3\frac{1}{2}$ hours. During this time the rate of the labored respirations fell from 200 or 100 to 50 or 60 per minute. In the superactive muscle fibers (Figs. 30 and 31) it is objectively evident that there is a substantial increase in size of the motor nerve plates. The clear ovoid areas both within the nerve plate and in the muscle substance are occupied by nuclei of the sole plate of Kühne.

The expanded nerve plates in the active muscle fibers have variable degrees of ameboid extensions of the terminal arborization of the axon. There appears to be a centrifugal extension of the terminal arborizations of the axon of the nerve plate, with increase of the surface area during muscle contraction; and a centripetal retraction of the processes, with decrease of the surface area, during relaxation of the muscle fiber.

Fractional contraction, in which some fibers are active, others at rest, appears to be the cause of these differences in structure. The active motor nerve plates have a greater number of dichotomous terminal divisions than the inactive ones. The nuclei of the granular sole plate of Kühne occupy the spaces between the divisions of the axon.

The diameter of the muscle fiber is not a reliable basis for classification of the muscle fibers (Figs. 22 and 23). Some wide fibers have coarse striations and some narrow fibers contain fine striations. This is in part dependent upon whether the muscle fiber is fixed in a state of isotonic or isometric contraction. In some fibers there is a simple alternation of dark Q and light J bands. The Q and J bands vary in width. In some there is a doubling of the Q band and in others the pattern of the so-called constant sarcomere structure of Z, J, Q, J, Z occurs. This pattern, however, is highly inconstant. There is, therefore, no constant number of so-called sarcomeres in this striped muscle.

These cross striations, therefore, in muscle, have variations in number and patterns such as the following: single Q bands (Figs. 2 and 3), double Q bands evenly spaced or increased in width in a geometric series radiating from the motor nerve plate (Figs. 3 and 8), J bands wider than the Q bands or the reverse (Figs. 17, 18 and 24), Q bands coarse (Figs. 6, 11 and 22), Q bands fine (Figs. 12, 13 and 23), Q bands arranged in a spiral, vernier shift or zigzag crossings (Figs. 2, 11 and 22), Q bands having a radiate pattern (Figs. 8 and 14), or the so-called sarcomeric pattern of Z, J, Q, J, Z (Figs. 12 and 23). There may be multiple patterns in a single muscle fiber (Figs. 8, 12 and 23). In cross sections there may be saturnine rings of the Q bands. It is readily apparent, therefore, that there are multiple variations of the cross striations in the intercostal muscle fibers. This may be confirmed at once by an inspection of the changeable patterns shown in Plates 1 to 18. There is no fixed pattern based on a unit of structure, the so-called sarcomere, in the cross striations of the intercostal muscle in the white rat. The internal structure of the intercostal muscle is as variable as its function.

There is dichotomous branching and anastomotic reticulation of the branchings of the axon in some of the nerve plates. In some plates there are terminal enlargements of the ramifications of the axon in the nerve plate. This enlargement of the nerve plate during increased functional activity is objective evidence of the transference of substance from the nerve to the muscle.

With progressive increase of ameboid expansion of the motor nerve plates there is a corresponding increase in number and fineness of closely spaced cross striations. The strongly contracted muscle fibers

have large motor nerve plates with two or more moniliform projections separated by wide spaces. With progressive increase in size of the motor nerve plate and increase of fineness of the dark and light transverse gratings, the contracted muscle fiber takes a lighter stain. There are many transitional stages and gradations between the coarsely and finely striated muscle fiber. There is a substantial ameboid protrusion of the processes of the nerve plate in various directions in the muscle substance which increases the surface area of the nerve plate. This is evidence that during life the muscle striae are not constant and rigid membranes.

There is an obliteration of the muscle striae below the plate on the lower muscle fiber shown in Figure 14, and a biconvex radiation of the muscle striae toward the left and right of the muscle fiber, away from the plate. These changes are likewise observed in nerve and muscle fixed *in situ* under normal tension by the terminal skeletal attachments. This pattern in the muscle is not one due to abnormal optical effects in photography, nor to crushing in teasing, nor to the pressure of the coverslip, since all these sources of error have been eliminated. This localized obliteration of the striations underlying the double motor nerve plate is observed at all levels of the focus. This motor plate is composed of two discrete components contributed by the dichotomous division of the single axon. There is a gradient in the fineness and spacing of the cross striations in the different phases of the functional activity of the muscle fiber in fractional contracture within a single muscle fiber.

When the motor nerve plate expands at one pole more than at the opposite one there is frequently a differential pattern of the related cross striations in a single muscle fiber (Figs. 15 and 18). At the upper, relatively non-expanded pole the striae are coarse and relatively regularly spaced. The oblong clear areas at the periphery of the nerve plates, in the granular sole plates of Kühne, are occupied by nuclei. There is a massive conduction of nerve substance into the axonal ramifications in the expanded motor nerve plates, stimulated by carbon dioxide.

The variable radiation and rhythmic replacement of the cross striations related to the functional activity of the motor nerve plate in the muscle fiber explains the differential morphology of the cross striations of the muscle (Figs. 16, 17 and 18). Irregular granular zones in the muscle fibers are related to motor nerve plates. The coarse striations at the ends of the muscle fibers are replaced by those related to an activity which appears to wipe out the striations underlying the nerve plates. This obliteration of the striations appears to occur at the

beginning of certain degrees of intensity of the propagated disturbance that radiates through the muscle fiber from the motor nerve plate. These are radiation areas of contracture in muscle, beginning at the nerve plate. Periodic areas of fine striations alternating with coarse ones are found in the muscle fiber (Figs. 19, 20 and 21). This subsequently results in a graded series of the spacing and width of the dark cross striations when fixed at the initial phases of expansion of the motor nerve plate in striated muscle. The pattern of the cross striations in muscle underlying the nerve plate therefore is highly inconstant.

The retracted plate (Fig. 32) has the clear ovoid regions occupied by the nuclei of the granular sole plate of Kühne close to the axonic nerve plate. With progressive explosive expansion of the plates by ameboid motion and sudden chemical changes these clear oval areas are projected into the muscle substance and at some distance from the nerve plate (Figs. 30, 33, 34, 35, 39, 48, 50, 51, 55, 58 and 63). There is a replacement of the clearness of the periodic structures in the muscle by the irregular granulation. The granules irregularly displace the cross striations. The ovoid clear areas in the muscle substance occupied by nuclei are surrounded by a dark granular radiation. In some places these clear oval areas may resemble vacuoles in both the nerve plate and the muscle.

Superactivity of hyperpnea evidently favors neurocladism, or dichotomous division of the branches of the axon in the nerve plate in intercostal muscle (Figs. 33, 34 and 35). The obstacles composed of nuclei of the sole plate of Kühne found in the crotch of the dividing terminal axon may condition the morphology of the expanding motor nerve plate during superfunctional ameboidism. There is a massive transmission of nerve substance into the axonal ramifications in the expanded motor nerve plates, stimulated by carbon dioxide. There is divisional ameboidism (Fig. 34) of the terminal axon, the projections of which grow out by superfunctional stimulation among and beyond the nuclei of the granular sole plate (Fig. 34).

These projections normally do not extend beyond the granular material of the sole plate of Kühne. The strong chemical, CO_2, acts as a neoformative stimulus to the production of the axonic branches of the motor nerve plate. There is a varicose or moniliform pattern to the axonic ramifications. When the axon is strongly stimulated and its terminals meet the resistance of nuclei and other protoplasmic obstacles, there results a precipitous and prolific projection of ramifications in the form of a bouquet (Figs. 8, 34, 35, 50 and 58). The dichotomous divisions extend between the nuclei of the dark granular sole plate of Kühne. The protoplasmic movement is the result of capillary

chemical change in concentration or composition, or both, of the axonic protoplasm, with a consequent modification of the internal pressure which extends along the lines of least resistance into the myoplasm.

It is this functional ameboidism of the terminal axonic bulbs that determines the pleomorphism of the motor nerve plates in striped muscle. It is evident that there is no constant morphology in the 88 . motor nerve plates shown in Plates 1 to 17.

The explosive patterns in muscle and nerve are not artefacts due to compression and teasing of the muscle fibers. They are found likewise in muscle fixed intact in the animal and then serially sectioned. The best evidence is obtained, however, when the continuity of the motor nerve plate and muscle fiber is maintained by teasing only gently and by preserving them together by the gold chloride method.

It is noteworthy that, in the direction in which the nuclei are projected from the periphery of the nerve plate into the muscle substance, there is a streamlike appearance in the sarcoplasm and a displacement of the regular pattern of cross striations by an irregular explosive arrangement of granules (Figs. 43, 44, 45, 46, 48, 50, 52, 54, 55 and 58). There is a direct continuity by a stream-line of granules between the clear ovoid and fusiform areas in the muscle substance and the granules of the sole plate of Kühne surrounding the axonic branches of the motor nerve plate (Figs. 44, 45, 48, 50, 52, 55 and 58).

The ultra-terminal collateral is observed (Fig. 62) as a fine nonmedullated branch of the axonal ramification in the typical, expanded, somatic motor nerve plate, and it ends in the same muscle fiber a short distance from the plate of origin. There is a terminal collateral which arises from the axon a short distance before it penetrates the sarcolemma and which ends as an independent small plate above the main nerve plate (Fig. 61). The protoplasmic extensions of the nerve plate may be related to the expansive effect of a strong electric stimulation or of any other strong chemical or mechanical stimulus.

Upon strong electrical stimulation there is a complete explosive disruption, dissolution and granular replacement of the arborizations of the axons in the two neighboring motor nerve plates (Fig. 67). This condition is found in 0.5 per cent of the motor nerve ends. There is likewise a displacement of the periodic pattern of muscle striae in this area of sudden and explosive disappearance of motor nerve plates by granular disorganization through strong electric stimulation. The nucleated ovoid clear areas are irregularly scattered in the myoplasm surrounding the ghostlike outline of the motor nerve plates. There is a massive transportation of nerve substance into the axonal branches in the expanded motor nerve plates, stimulated by electricity (Figs.

60, 61, 62, 63, 64, 65 and 66). There is an accompanying explosive perturbation of the related protoplasm of the striped muscle fiber. The surface area of the strongly stimulated motor nerve plate (Fig. 63) is greatly increased over that of the relatively resting nerve plate (Fig. 59). This difference in size of the plates in the same stimulated nerves and muscle fibers is due to fractional contraction. The problem of the transmission mechanism, in which the impulse in one axon with many terminals activates some nerve ends in muscle while others remain relatively inactive, is still unsolved. It is a fact that one axon may divide and terminate in two nerve ends in two neighboring muscle fibers. One nerve end may be expanded while the other is retracted. The nature of this alternating, shunting mechanism in the conduction of the nerve impulse resulting in differential rest and motion of the fibers in a single muscle is still unknown.

Specific structural changes produced by the strong electric current in muscle fibers were best studied in the winter frog, or in the summer frog after it had been in the refrigerator at 6° to 8° C. for 48 hours (Figs. 68 and 69). The nuclei in the muscle fibers of either naturally or artificially hibernating frogs became centrally located in the muscle fiber. When contracture was produced by electricity the myoplasm in the single muscle fiber was arranged in a series of alternating condensed nodes and rarefied internodes. The nuclei were compressed in the nodes of condensation and stretched in the internodes of rarefaction. These condensed nodes and rarefied internodes were assumed to be the two physical components of a train of longitudinal waves of compression that accompany the variable capillary chemical reactions in the microcapillary muscle fiber.[13] The periodicity of the structure of both the normal and abnormal muscle fibers was dependent upon both the physical and chemical factors associated during changes in the capillary chemistry. These histologic findings confirm the physiologic observations on muscle contracture reviewed by Gasser [29] and those of Tower [30] on the reaction of muscle to denervation.

SUMMARY

Evidence is presented that functional ameboidism determines the pleomorphism of both the normal and abnormal motor nerve plates in intercostal muscle. The respiratory rate in rats subjected to carbon dioxide was accelerated from 30 to 200 per minute with labored breathing and hyperpnea. Direct evidence is presented, based on the effect of hyperpnea by carbon dioxide, that the motor nerve plate physically vibrates during functional activity. It expands when the muscle fiber contracts and constricts or retracts when the muscle fiber relaxes.

The retracted motor nerve plates with wide, short projections are related to coarse, widely spaced cross striations, whereas expanded nerve plates with thin, elongated and moniliform processes are longer and separated by wider meshes than the retracted ones and are related to fine, closely spaced cross striations (Fig. 17). Over 60 per cent of the expanded motor nerve plates in the intercostal muscle in which the rate of transmission of the nervous impulses was accelerated by carbon dioxide became twice the size of the relatively normal plates. Their processes were longer and separated by wider meshes than those of the normal controls. This morphologic expansion and extension of the terminal arborization in the nerve plate by ameboid motion under the stimulus of the demand of increased functional activity evidently favors the transmission of the nerve impulse to the receptive muscle substance by increase of the surface area of the plate.

The differences in the histologic structure of motor nerve plates and muscle cross striations are arranged in a graded series and evidently are determined by the active and inactive fibers in the fractional contraction of the same muscle. These structural changes favor the chemical theory of impulse transmission. Ameboid motion explains the pleomorphism of the motor nerve plates fixed in the closely related active and inactive muscle fibers of a muscle.

Strong stimulation by carbon dioxide and electricity produces a pathologic explosion of the ameboid structure of the motor nerve plates with turbulence of the muscle cross striations. The pattern of the cross striations in intercostal muscle is as variable as that of the internal energy associated with the capillary chemistry confined in the microcapillary muscle fibers. There is no constant unit of structure, such as the so-called sarcomere, in the intercostal muscle fiber. Therefore, (1) functional ameboid motion determines the pleomorphic structure of the motor nerve plates, and (2) changeable capillary chemistry determines the pleomorphic nature of the variable periodic structure in striped muscle.

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DESCRIPTION OF PLATES

All of the untouched photomicrographs, Plates 35 to 51, are from the teased right intercostal muscle fibers of the white rat, consisting of: 1, relatively normal muscle and nerve plates; 2, those subjected to carbon dioxide stimulation; and 3, those electrically stimulated through the spinal cord by the ordinary alternating house current, 120 volts, 1 ampere, for 1 minute.

Plates 35 to 38 are photographs of the teased right intercostal muscles of rat no. 25. It is important to note that Plates 39 to 51 are photographs of the teased right intercostal muscles and contain pleomorphic motor nerve endings from the same animal, rat no. 57. All were prepared by the gold chloride technic. All figures in Plates 36 to 51 are magnified 750 ×. In Plate 35, Figure 1 is magnified 300 ×; Figure 2 is magnified 750 ×.

PLATE 35

FIG. 1. Sprays of medullated nerve fibers and motor end-plates in normal teased intercostal muscle fibers, white rat no. 25. The darkly stained relaxed muscle fibers have coarse, widely spaced cross striations, whereas the lightly stained contracted muscle fibers have fine, closely spaced striae. There are many transitional stages between these two extremes. The upper pair of motor nerve plates are derivatives of a single axon; one branch terminates in a retracted motor plate in a relaxed muscle fiber, the other terminates in an expanded nerve plate in a contracted muscle fiber. The middle pair of nerve plates is composed of one retracted and one expanded plate, derived from a single axon. These normal teased intercostal muscles were obtained before the rat was subjected to CO_2.

FIG. 2. Normal teased intercostal muscle fibers, white rat no. 25, gold chloride. The central darkly stained, relaxed muscle fiber has coarse, widely spaced cross striations, whereas the expanded nerve plates in the two contracted fibers have variable degrees of fineness and spacing of the related cross striations. The expanded nerve plates in the active muscle fibers have variable degrees of ameboid extensions of the terminal arborization of the axon. There appears to be a centrifugal extension of the terminal arborizations of the axon of the nerve plate, with increase of the surface area during muscle contraction; and a centripetal retraction of processes, with decrease of the surface area, during relaxation of the muscle fiber. Fractional contraction, or some fibers active with others at rest, appears to be the cause of these differences in structure. The active motor nerve plates have a greater number of dichotomous terminal divisions than the inactive ones. There are 15 dark striations related to the retracted motor plate on the middle muscle fiber and 24 related to the expanded nerve plate on the lower muscle fiber.
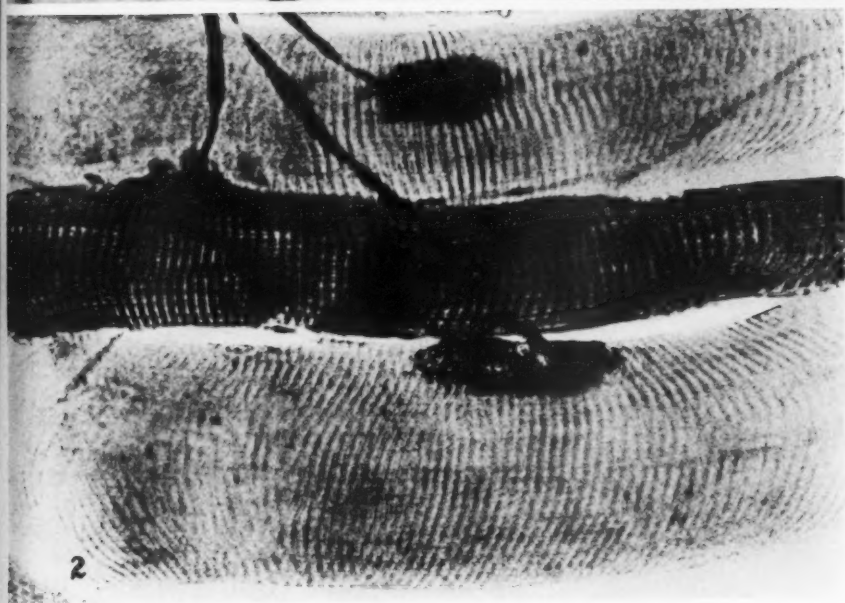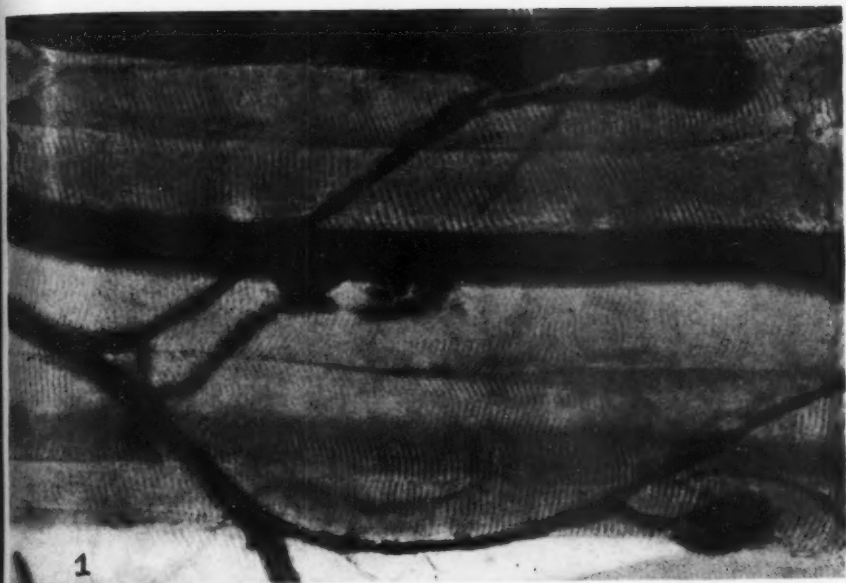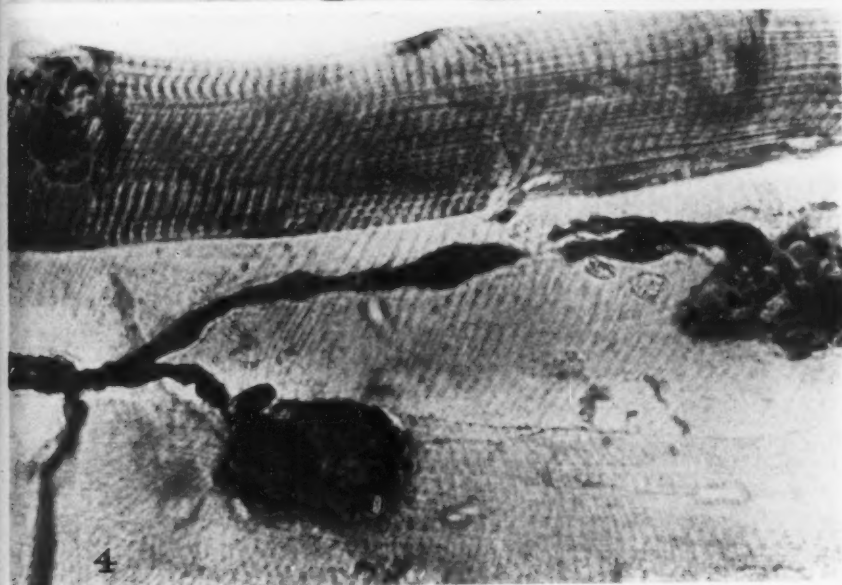
Carey

Explosive Ameboid Motion in Nerve Plates

PLATE 36

FIGS. 3 and 4. Normal coarsely and finely striped muscle fibers. There is an irregular radiation pattern of the fine, closely spaced cross striations in relation to the expanded ending in the lower muscle fiber (Fig. 3). These cross striations become coarser and more widely spaced with increase of the distance from the nerve plate toward the left of this fiber. Variations in the end-plates and muscle striae are also observed in Figure 4. The diameter is not a reliable basis for classification of muscle fibers. Some wide fibers have coarse striations and some narrow fibers contain fine striations. This is in part dependent upon whether the muscle fiber is fixed in a state of isotonic or isometric contraction. In some fibers there is a simple alternation of dark Q and light J bands. The Q and J bands vary in width. In some plates there is a doubling of the Q band and in others the pattern of the so-called constant sarcomere structure of Z, J, Q, J, Z. This pattern, however, is highly inconstant. There is, therefore, no constant number of so-called sarcomeres in this striped muscle. The active motor nerve plates have a greater number of axonal ramifications than the inactive ones. There are 14 dark striations related to the retracted motor plate on the upper muscle fiber and 29 dark striations related to the expanded plate on the lower muscle fiber (Fig. 3).

PLATE 36

Carey

Explosive Ameboid Motion in Nerve Plates

PLATE 37

FIGS. 5 and 6. Normal relaxed dark and contracted light muscle fibers containing retracted and expanded motor end-plates, respectively. There is a granular obliteration of the cross striations to the left of the upper light muscle fiber in Figure 5 and to the right of the upper light muscle fiber in Figure 6. The wide dark bands with relatively narrow light bands are clearly seen in the narrow, resting and dark lower muscle fiber (Fig. 6). With progressive increase in intensity of contraction the dark Q band narrows, the light J band broadens, the Z band appears and a duplex Q band occurs. The pseudopodia of the nerve plate in the upper fiber (Fig. 6) are dichotomously branched into tuning-fork-like processes. The nuclei of the granular sole plate of Kühne are found within the crotch of the divisions of the axon. The active motor nerve plates have a greater number of axonal branches and a greater number of related cross striations than the inactive nerve plates.
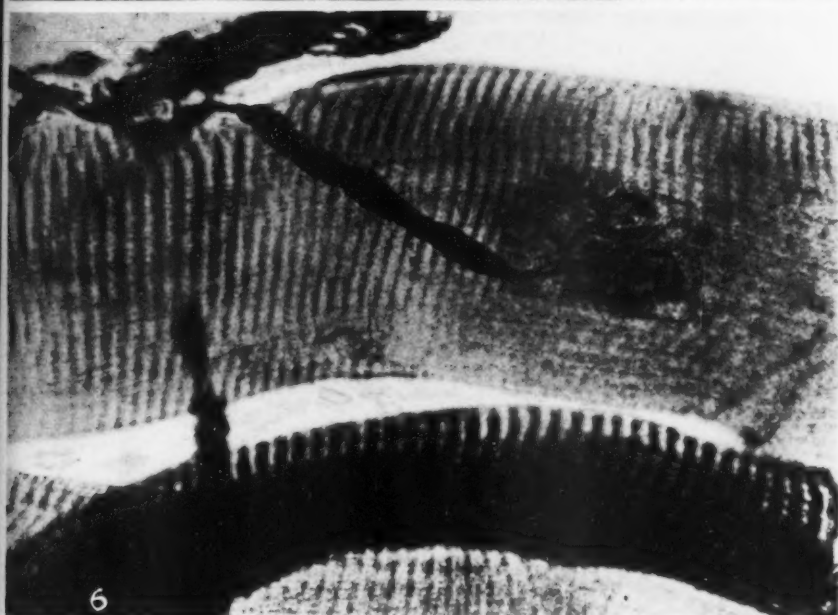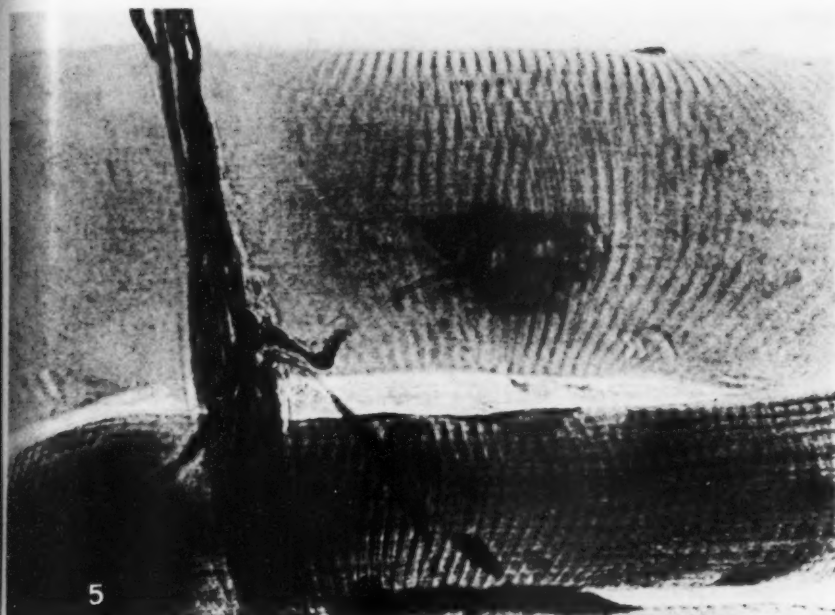
PLATE 38

FIG. 7. The teased right intercostal muscles and motor nerve ends (Figs. 7 and 8) are from the same animal. One normal coarsely striated muscle fiber lies between two finely striated ones. The retracted nerve plate on the coarsely striped muscle fiber has a dark granular sole plate of Kühne. This sole plate has undergone progressive dissolution in the two expanded nerve plates. The oval clear areas at the left and right poles of the lower nerve plate are occupied by nuclei of the granular sole plate of Kühne.

FIG. 8. Expanded motor nerve plate and muscle fiber stimulated for 1 minute with CO_2. This expanded nerve plate appears to be formed during superfunctional activity by the streaming out of the protoplasm into the sarcoplasm centrifugally from the proximal axonic axis. There is dichotomous branching and anastomotic reticulation of the branchings of the axon in the nerve plate. In some places there are terminal enlargements of the ramifications of the axon in the nerve plate. This enlargement of the nerve plate during increased functional activity is objective evidence of the transference of substance from the nerve to the muscle. The dark Q bands are double and form an irregular radiation pattern around the nerve end. To the left of the nerve end there is an obliteration of the striated pattern with the replacement of the cross striations by granules. The vascular loop surrounding the motor nerve plate, called *confluens capillorum* by Wilkinson,[20] is clearly evident. The vessels that form the loop become more dilated than normally upon strong stimulation by either CO_2 or electricity. The motor nerve plate stimulated by CO_2 has a greater number of axonal arborizations than the inactive one. This stimulated plate appears to possess the capacity of neurocladism in the terminal turgescent bulb. This is the resultant of divisional ameboidism of the terminal nerve bulb upon strong stimulation. The resistance presented by the sole-plate nuclei of Kühne evidently favors the division of the protoplasmic streaming of the terminal pseudopodia of the axonic bulb. There are 11 dark striations related to the retracted nerve plate on the middle muscle fiber (Fig. 7) and 35 dark striations related to the expanded nerve plate (Fig. 8).

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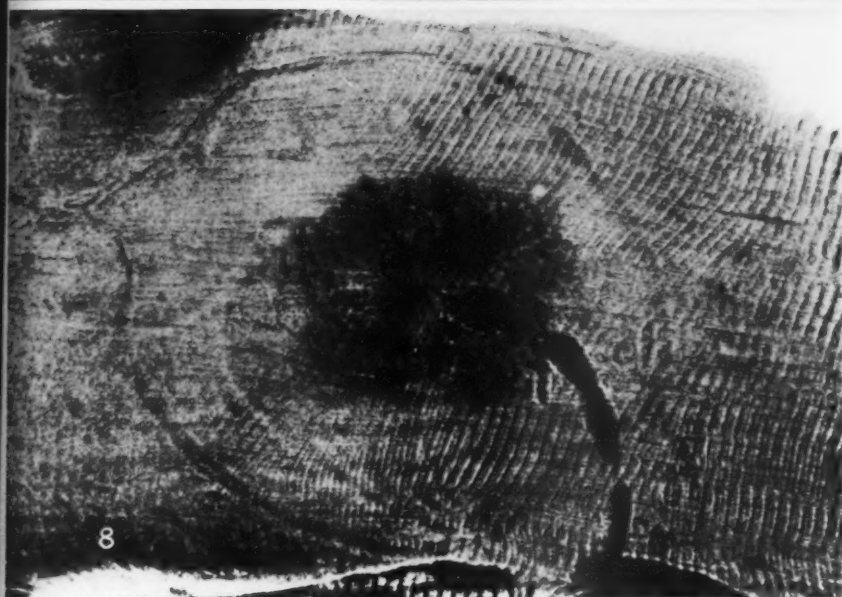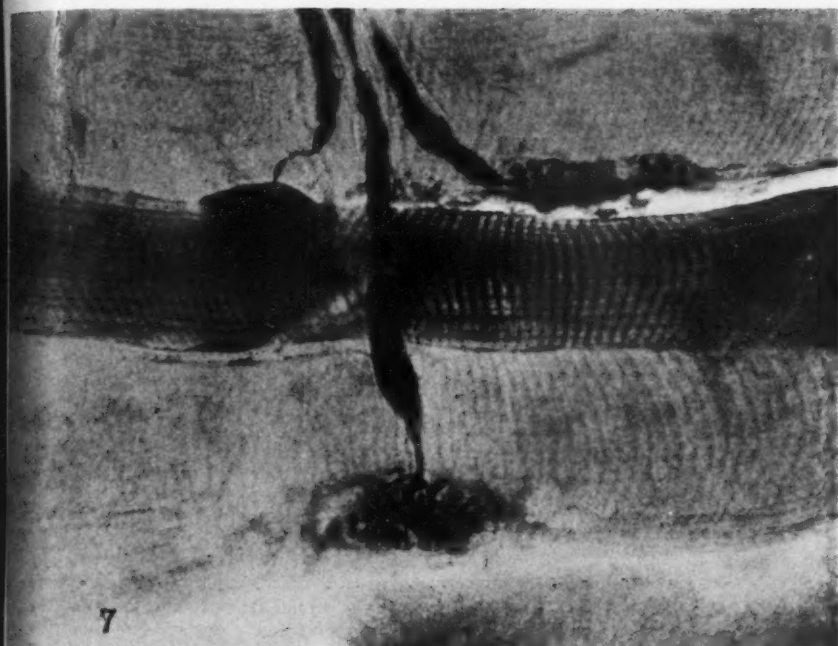
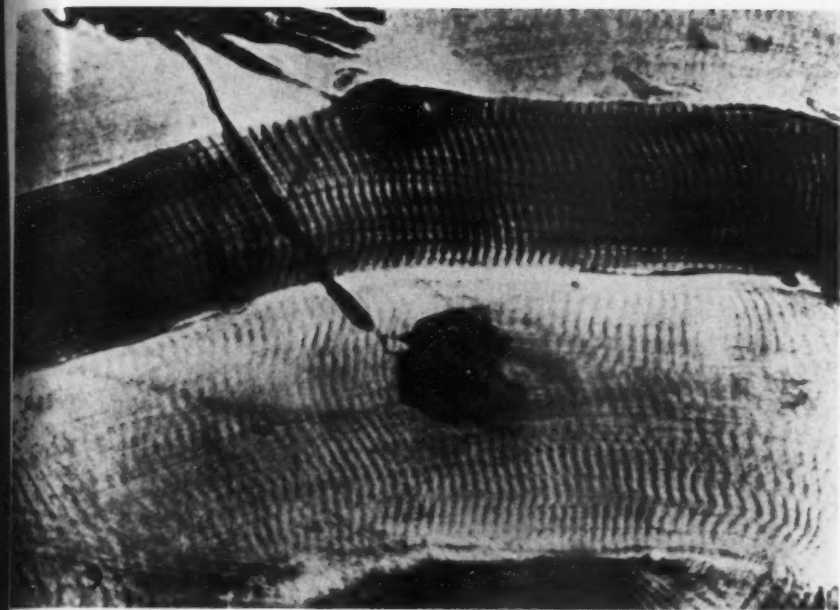Carey   Explosive Ameboid Motion in Nerve Plates

PLATE 39

FIG. 9. Normal dark and light intercostal muscle fibers. The narrow dark and re-
laxed muscle fiber has coarse darkly stained cross striations and a retracted
motor nerve plate surrounded by Kühne's granular sole plate. The light muscle
fiber has fine closely spaced cross striations close to the slightly expanded motor
nerve plate. The granular sole plate of Kühne is undergoing dissolution. The
cross striations vary in fineness and spacing and in the relative proportions of
space occupied by the dark and light bands.

FIG. 10. Intercostal muscle fibers, stimulated for 1 minute with CO_2. With progres-
sive increase of ameboid expansion of the motor nerve plates there is a cor-
responding increase in number and fineness of closely spaced cross striations.
The strongly contracted muscle fibers have large motor nerve plates with two
or more moniliform projections separated by wide spaces. With progressive
increase in size of the motor nerve plate and increase of fineness of the dark
and light transverse gratings the contracted muscle fiber takes a lighter strain.
There are many transitional stages and gradations between the coarsely and
finely striated muscle fiber. There is a substantial ameboid protrusion of the
processes of the nerve plate in various directions in the muscle substance which
increases the surface area of the nerve plate.

PLATE 39

Carey

Explosive Ameboid Motion in Nerve Plates

PLATE 40

FIGS. 11, 12 and 13. Progressive expansion of the motor nerve plates and change in periodic structure of the cross striations in the intercostal muscle stimulated by CO_2 for 1 minute. There appears to be a centrifugal flow of substance or protrusion of the axonic terminals of the nerve plate into the muscle substance from the retracted state (Fig. 11) to that of the variable degrees of expansion (Figs. 12 and 13). Ameboid motion is the changeable play in the centrifugal expansion and centripetal retraction of the axonic pseudopodia. The coarse muscle striae are related to the retracted plate and the fine striae to the expanded ones. There is a richly branched pseudopodial network in the lower expanded nerve plate (Fig. 13). The clear ovoid areas within and at the periphery of the nerve plates are occupied by the nuclei of the sole plate of Kühne. The irregular radiation pattern of the striae defy description in words. There is a massive transference of nerve substance into the axonal ramifications in the expanded motor nerve plates stimulated by CO_2.

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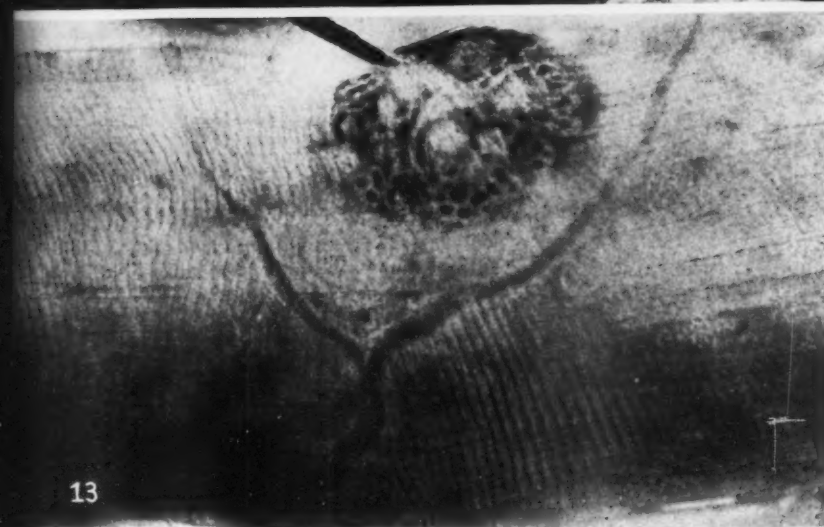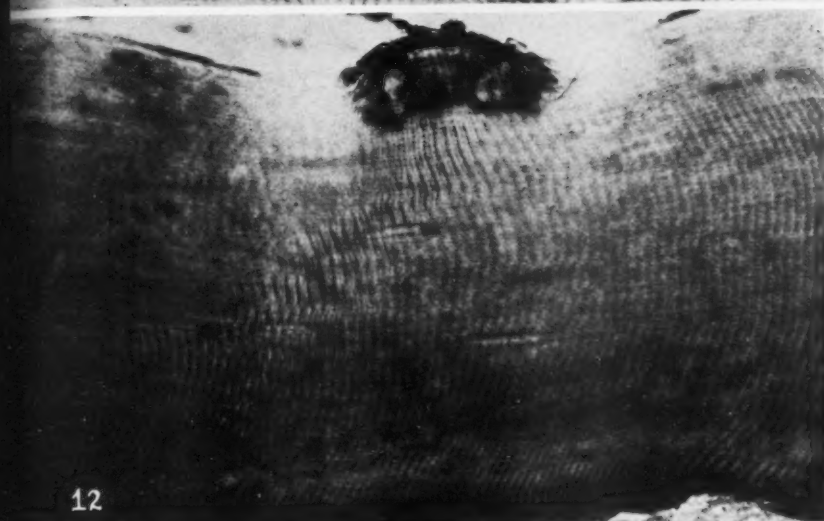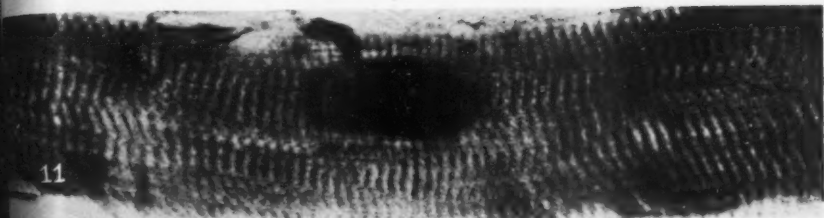
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PLATE 41

FIGS. 14 and 15. Intercostal muscle fibers and motor end-plate stimulated to hyper-
pnea with CO_2 for 1 minute. There is a dichotomous division of the axon be-
fore it terminates on the nerve plate of the lower muscle fiber (Fig. 14). There
is an obliteration of the muscle striae below this plate, and a biconvex radia-
tion of the muscle striae toward the left and right in the muscle fiber. These
changes are observed in nerve and muscle fixed *in situ* under normal tension
of the terminal skeletal attachments. This pattern in the muscle is not one
due to abnormal optical effects in photography, to crushing in teasing nor to the
pressure of the coverslip. This localized obliteration of the striations under-
lying the double motor nerve plate is observed at all levels of the focus. This
motor plate is composed of two discrete components contributed by the dichot-
omous division of the single axon. There is a gradient in the fineness and
spacing of the cross striations in the different phases of the functional activity
of the muscle fiber in fractional contracture. There is a polar connection of the
medullated axon with the motor nerve plate (Fig. 15). The widened dark axon
is separated from the fine dark linear neurilemma by the clear medullary
sheath which fails to take the gold deposit. The neurilemma is continuous with
the sarcolemma. Immediately underneath, to the right of and below each
nerve plate, the fine dark striations are irregular and closely spaced. Above the
nerve plates the dark cross striations are coarse and widely spaced. There is a
faint Z band between the coarse Q bands, bounded on each side by half J bands.
This Z band is highly inconstant in the highest magnifications of the muscle
fiber. When the motor nerve plate expands at one pole more than at the oppo-
site one there is frequently a differential pattern to the related cross striations
in a single muscle fiber. At the lower expanded pole (Fig. 15) the striae are
fine and irregular. At the upper relatively non-expanded pole the striae are
coarse and relatively regularly spaced. The oblong clear areas at the periphery
of the nerve plates, in the granular sole plates of Kühne, are occupied by nuclei.
There is a massive conduction of nerve substance into the axonal ramifications
in the expanded motor nerve plates, stimulated by CO_2.
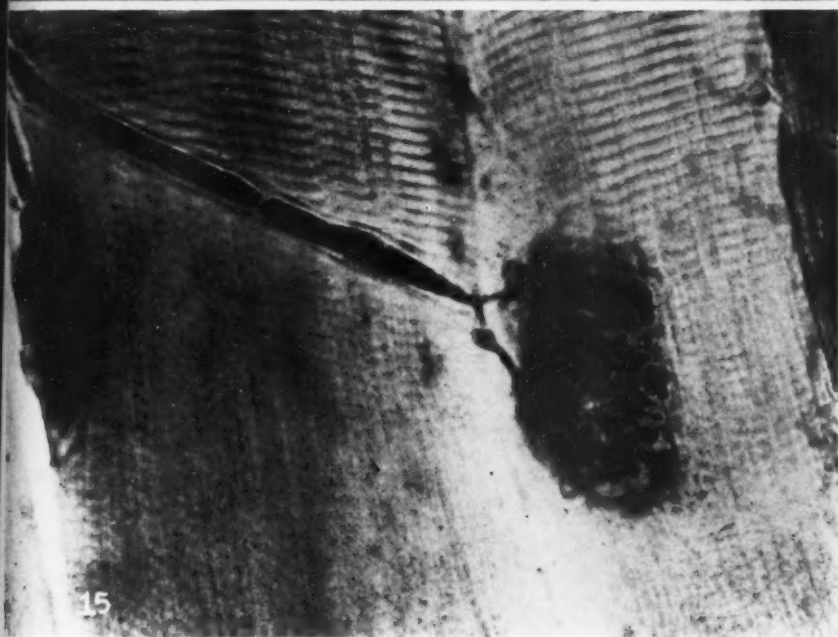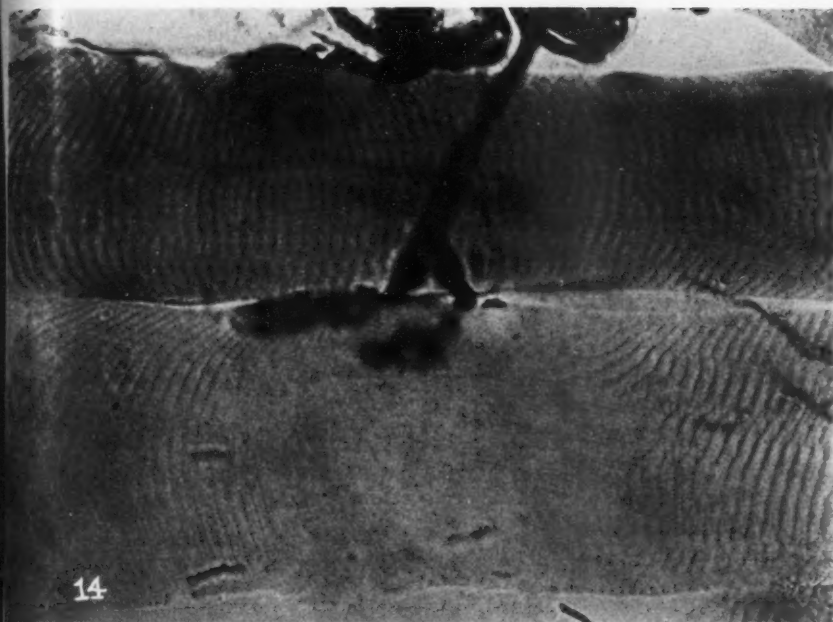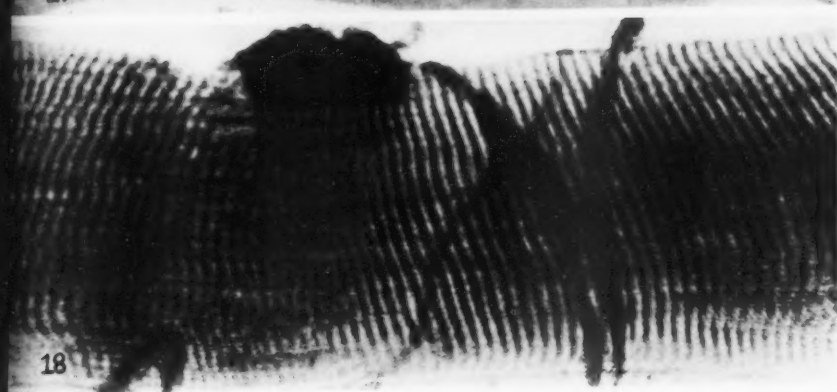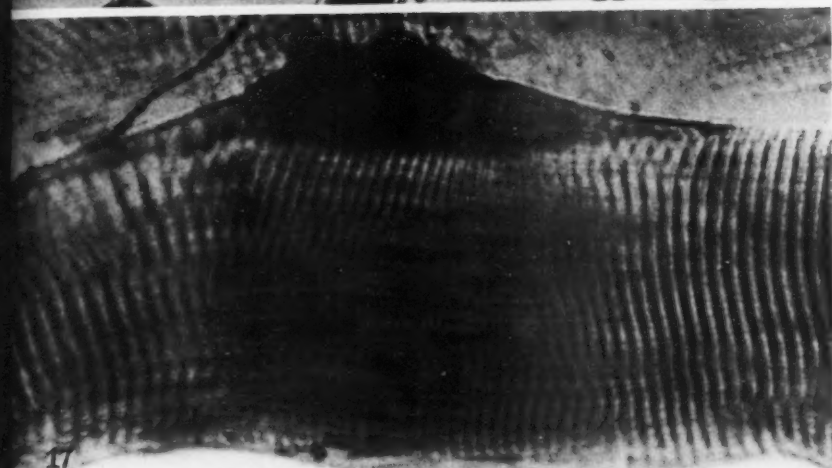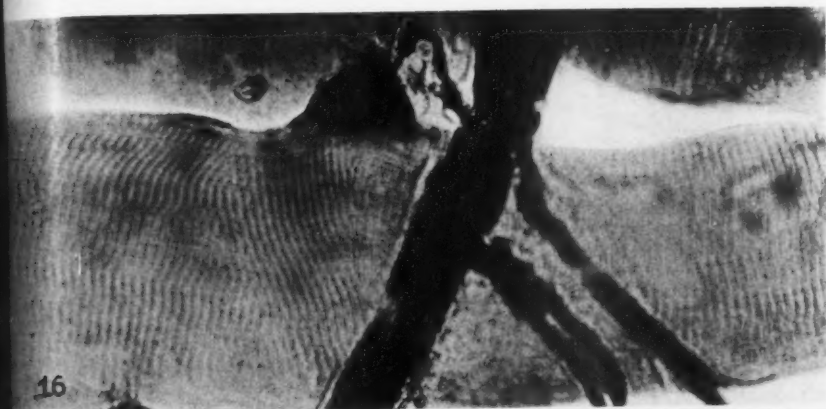
PLATE 41

Carey

Explosive Ameboid Motion in Nerve Plates

PLATE 42

FIG. 16. The hypolemmal motor nerve plate in profile view is related to a pattern of muscle striae called the typical *Noniusfelder* of Heidenhain. The variable radiation and rhythmic replacement of the cross striations related to the functional activity of the motor nerve plate in the muscle fiber may explain the differential morphology of the cross striations of muscle (Figs. 16, 17 and 18). This is from the third left intercostal muscle, 1 minute after exposure in an atmosphere of CO_2.

FIGS. 17 and 18. Irregular granular zones in the muscle fibers are related to motor nerve plates. The coarse striations at the ends of the muscle fibers are replaced by those related to an activity which appears to wipe out the striations underlying the nerve plates. This obliteration of the striations appears to occur at the beginning of certain degrees of intensity of the propagated disturbance that radiates through the muscle fibers from the motor nerve plate. These are radiation areas of contracture in muscle, beginning at the nerve plate. This subsequently results in a graded series of the spacing and width of the dark cross striations when fixed at the initial phases of expansion of the motor nerve plate in striated muscle. The pattern of the cross striations in muscle underlying the nerve plate is highly inconstant.
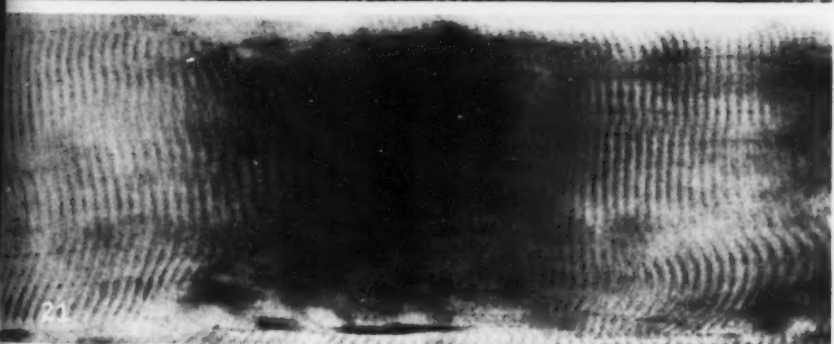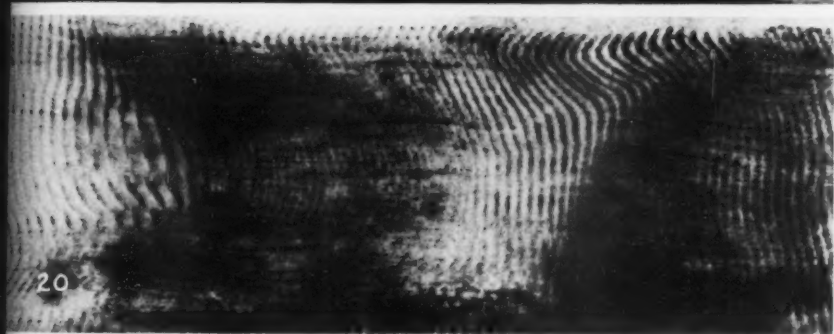
Explosive Ameboid Motion in Nerve Plates

PLATE 43

FIG. 19. Two teased right intercostal muscle fibers, stimulated with CO_2. The nerve plates are in profile and the related muscle striae are fine and closely spaced to the right, and coarse and widely spaced to the left of the plate. This differential structure of the muscle striae is related to differential expansion of the nerve plate.

FIGS. 20 and 21. Right intercostal muscle fibers with periodic groups of fine striations alternating with coarse striae. To the right of the upper muscle fiber (Fig. 20) each S-shaped, coarse stria divides below into two fine striae. These nodes of contracture composed of fine striations are radiated in groups from the irregularly active motor nerve plates.
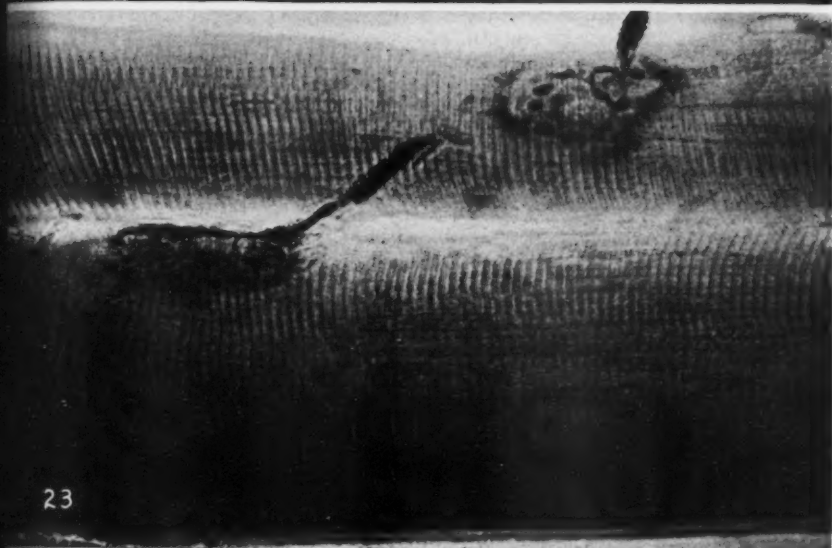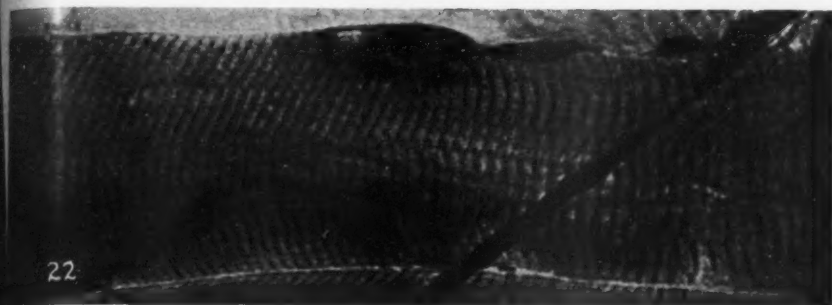
PLATE 44

FIG. 22. Intercostal muscle fiber; hyperpnea by stimulation for 1 minute with CO_2. The retracted motor nerve plate, surrounded by the granular sole plate of Kühne, has fine, closely spaced cross striations related toward the left, and wide, closely spaced striae toward the right of the muscle fiber.

FIG. 23. The upper motor nerve plate has a circular outline, whereas the lower one is more expanded. The cross striations have in some places a doubling of the Q band, and in others a Z, J, Q, J, Z pattern. In still other regions the striae have a spiral pattern.

FIG. 24. Toward the right of the muscle fiber the dark Q bands alternate with the light J bands. Toward the left there is an irregular criss-cross pattern. There are four branches of the single axon in the motor nerve plate. Toward the right the ovoid space is occupied by a nucleus of the granular sole plate of Kühne. The muscle fibers of this plate illustrate the varying coarseness and fineness of the cross striations and related degrees of retraction and expansion of the motor nerve plates, respectively.
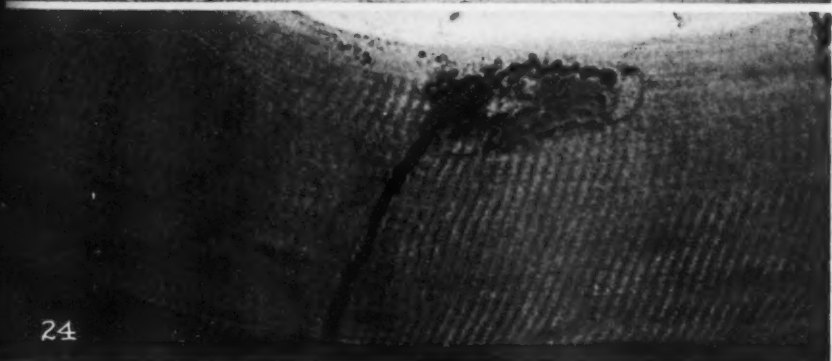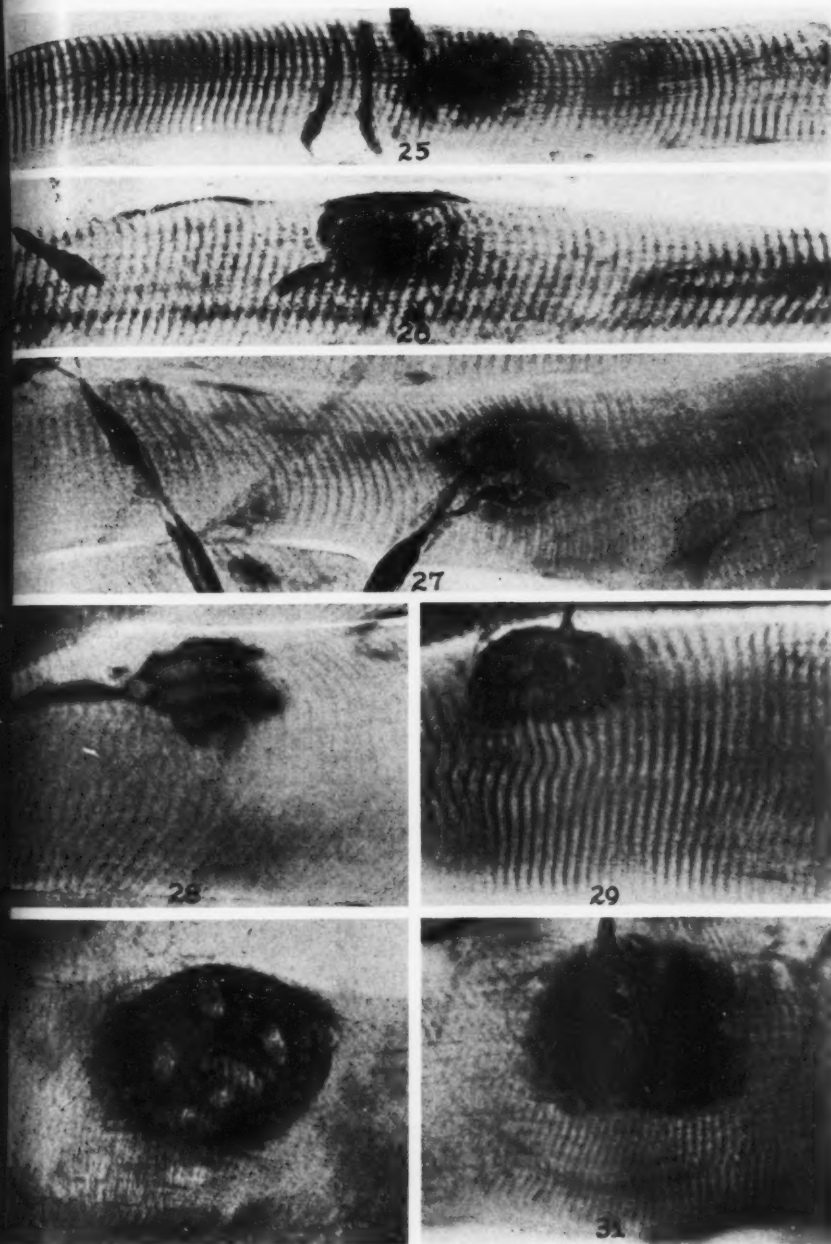
Explosive Ameboid Motion in Nerve Plates

PLATE 45

Figs. 25 to 31. Pleomorphism by ameboid changes of axonic branches in motor nerve plates in teased intercostal muscle fibers; hyperpnea by CO_2 stimulation for 1 minute. The retracted and slightly expanded nerve plates are surrounded by the dark granular sole plate of Kühne (Figs. 25, 26 and 27). This granular sole plate disappears in the expanded plates of the highly active muscle fibers. There are 11 dark cross striations related to the retracted nerve plate in the relaxed muscle fiber (Fig. 25). There are 32 dark cross striations related to the expanded motor nerve plate in the active muscle fiber (Fig. 31). This differential numerical relationship of the muscle striae related to the retracted and expanded nerve plates has a definite relationship to physiologic and pathologic functions at the myoneural junction. In the superactive muscle fibers (Figs. 30 and 31) it is objectively evident that there is a substantial increase in size of the motor nerve plates. The clear ovoid areas, both within the nerve plate and in the muscle substance, are occupied by nuclei of the granular sole plate of Kühne. The vascular loop surrounding the motor nerve plate, called *confluens capillorum* by Wilkinson, is clearly evident. There is a massive conduction of nerve substance into the axonal ramifications in the expanded motor nerve plates, stimulated by CO_2. The ultra-termination of the axonic pseudopodia of the motor nerve end with muscle is highly variable. There is no constant Z or Krause membrane, or other periodic structure, or periterminal network.
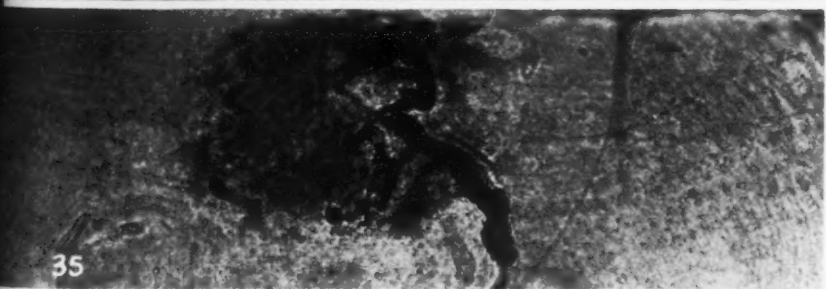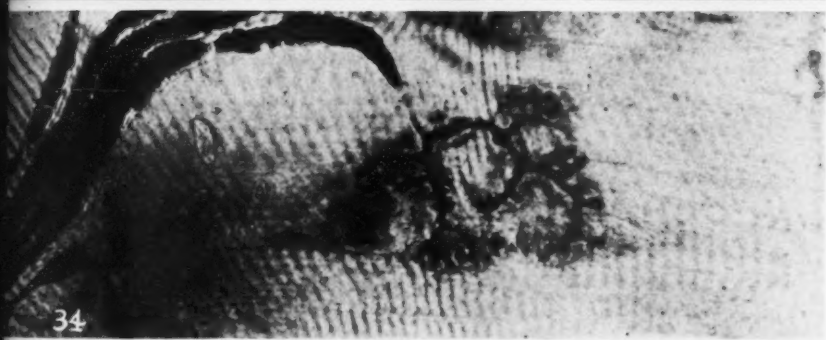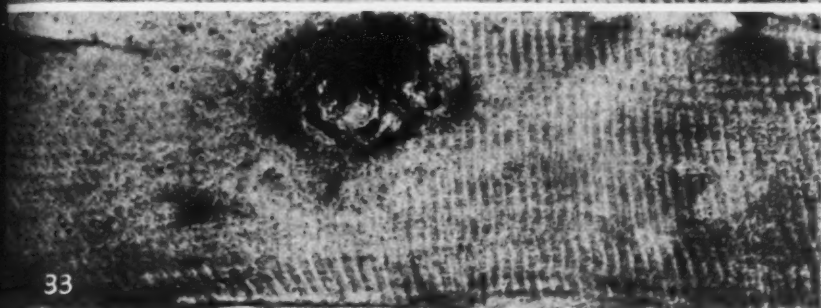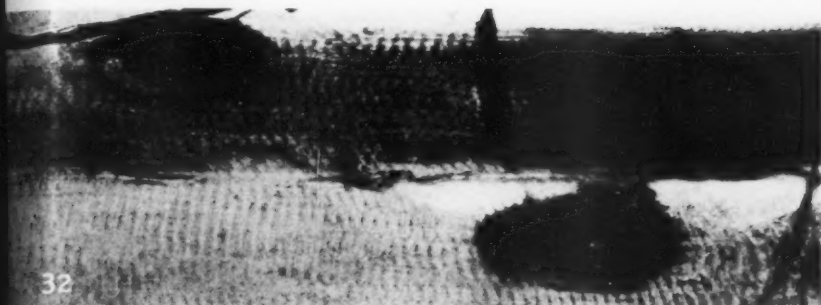
PLATE 46

FIGS. 32 to 35. Pleomorphism by ameboid changes of axonic branches in motor nerve plates in teased muscle fibers, hyperpnea by CO_2 stimulation for 1 minute. The retracted plate (Fig. 32) has the clear ovoid places occupied by the nuclei of the granular sole plate of Kühne close to the nerve plate. With progressive explosive expansion of the plates by ameboid motion and sudden chemical changes these clear oval areas are projected into the muscle substance and at some distance from the nerve plate (Figs. 33, 34 and 35). There is a replacement of the clearness of periodic structures in the muscle by the irregular placement of granules. The ovoid clear areas in the muscle substance occupied by nuclei are surrounded by a dark granular radiation. In some places these clear oval areas may resemble vacuoles in both the nerve plate and in the muscle. Superactivity evidently favors neurocladism, or dichotomous division of the branches of the axon in the nerve plate (Figs. 33, 34 and 35). The obstacles composed of nuclei of the granular sole plate of Kühne found in the crotch of the dividing terminal axon may condition the morphology of the expanding motor nerve plate during superfunctional ameboidism. There is a massive transmission of nerve substance into the axonal ramifications in the expanded motor nerve plates when stimulated by CO_2. There is divisional ameboidism (Fig. 34) of the terminal axon, the projections of which grow out by superfunctional stimulation among and beyond the nuclei of the granular sole plate of Kühne. These projections normally do not extend beyond the granular material of the sole plate of Kühne. The strong chemical, CO_2, acts as a neoformative stimulus to the production of the axonic branches of the motor nerve plate. There is a varicose, or moniliform, pattern to the axonic ramifications. When the axon is strongly stimulated and its terminals meet the resistance of nuclei and other protoplasmic obstacles there results a precipitous and prolific projection of ramifications (Fig. 34). The dichotomous divisions extend between the nuclei of the dark granular sole plate of Kühne. The protoplasmic movement is the result of capillary chemical change in concentration or composition, or both, of the axonic protoplasm with a consequent modification of the internal pressure which extends along the lines of least resistance.
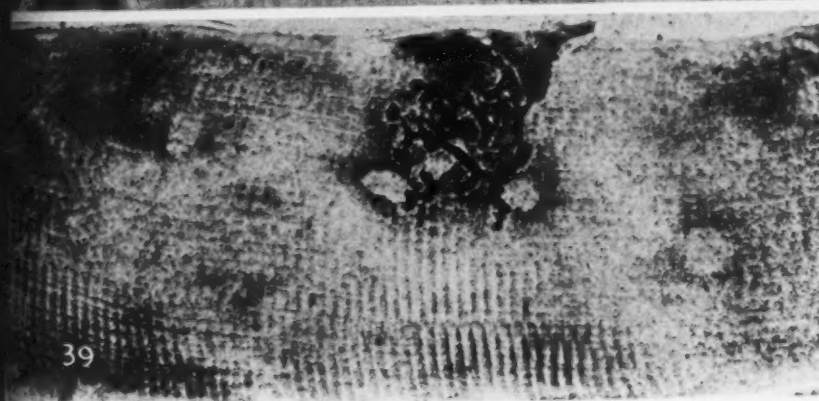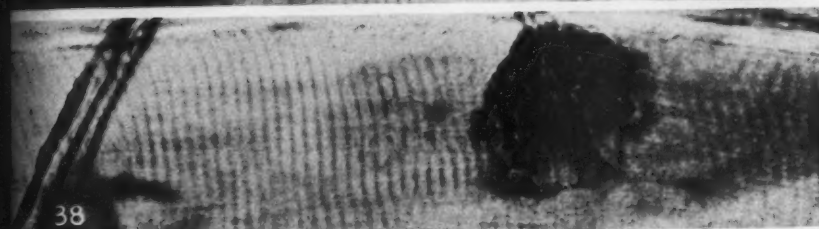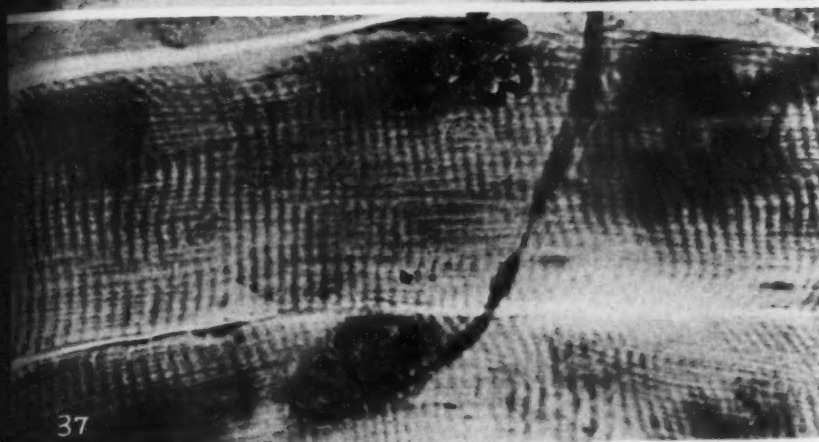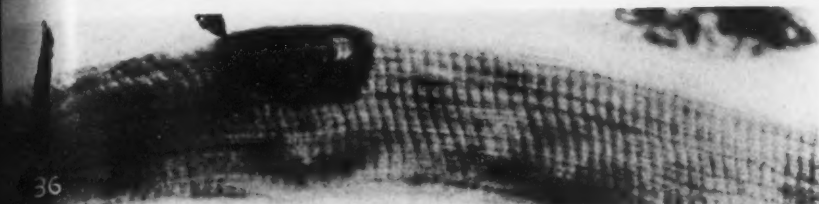
PLATE 46

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Explosive Ameboid Motion in Nerve Plates

PLATE 47

FIGS. 36 to 39. Pleomorphism of retracted and expanded motor nerve plates in right intercostal muscle, after 1 minute of hyperpnea by CO_2 stimulation. The relatively slightly active, inactive and retracted motor end-plates have short, wide, coarse fronds (Figs. 36 and 37). The superactive and expanded motor nerve plates have long, narrow pseudopodia, or terminal branches, of the axon in the nerve plate. Concomitant with the explosive expansion of the nerve plate (Figs. 33, 39, 50, 58 and 63) there appears to be an explosive replacement of the muscle striae by granules. At great distances to the left and right of the nerve plate and in the granulation of the muscle substance, there are found clear areas which contain the unstained nuclei. These nuclei have been projected into the muscle substance, and away from their close peripheral position to the nerve plate. These nuclei in the muscle substance have the same morphology, continuity and relationship to the surrounding radiation of granules as those that still maintain the close position in the intact sole plate of the motor axonic nerve plate. These explosive patterns in muscle and nerve are not artefacts due to compression and teasing of the muscle fibers. They are found likewise in muscle fixed intact in the animal and then serially sectioned. The best evidence is obtained, however, when the continuity of the motor nerve plate and muscle fiber is preserved by gently teasing and preserving them together by the gold chloride method. There is a massive transportation of nerve substance into the axonal ramifications in the expanded motor nerve plates, stimulated by CO_2.
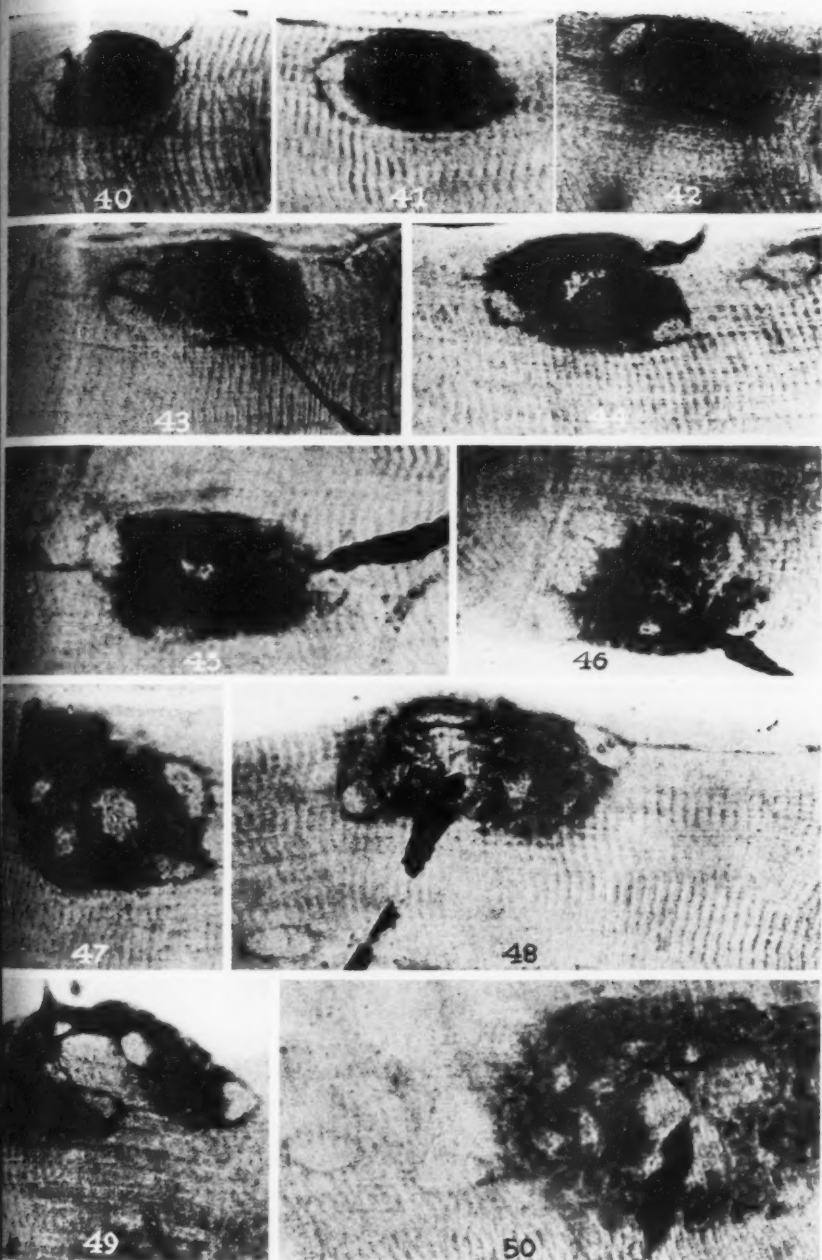
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Explosive Ameboid Motion in Nerve Plates

PLATE 48

FIGS. 40 to 50. Pleomorphism of the motor nerve plates, by superfunctional ame-
boidism of the axonic terminals, in teased right intercostal muscle fibers;
hyperpnea due to CO_2 stimulation for 1 minute. The clear ovoid nucleated
areas, which appear like vacuoles, are both closely related to the periphery of
the nerve plates, within the granular sole plate of Kühne, and displaced at
some distance in the muscle substance by some explosive force related to the
sudden expansion of the motor nerve plate. Note that in the direction in which
the nuclei are projected from the periphery of the nerve plate into the muscle
substance there is a streamlike effect in the sarcoplasm and a replacement of
the regular pattern of cross striations by an irregular arrangement of granules
(Figs. 43, 44, 45, 46, 48 and 50). There is a direct continuity by a stream-
line of granules between the clear ovoid and fusiform areas in the muscle sub-
stance and the granules of the sole plate of Kühne surrounding the axonic
branches of the motor nerve plate (Figs. 44, 45, 48 and 50). There is a mas-
sive conduction of nerve substance into the axonal ramifications in the ex-
panded motor nerve plates, stimulated by CO_2. The motor nerve plates are
morphologically lawless and become explicable only on the physiologic and
pathologic basis of the dynamics of functional ameboidism. The retraction of
the pseudopods of axonic arborization toward the base of the stem forms a
wide terminal bulb or cone (Fig. 40). This terminal bulb has wide, short
lobes. The pleomorphism and acute hypertrophy of the motor end-plates are
produced by ameboid expansions and extracentral ramifications and reticula-
tions of the numerous axonic projections under the strong stimulus of CO_2.
The nerve end-plate is a plastic mass undergoing constant change in chemical
composition and concentration, with resultant morphologic changes from a
small or large bud (Fig. 40) to a large bouquet with extensive ramifications
(Fig. 50). The tuberous arborizations of the sprouting axon become pro-
gressively thinner with varicosities at the bifurcations and terminals of the
processes. Accumulation of protoplasm or intumescence occurs at the end of
each branch of the axon. The branches take different configurations and ter-
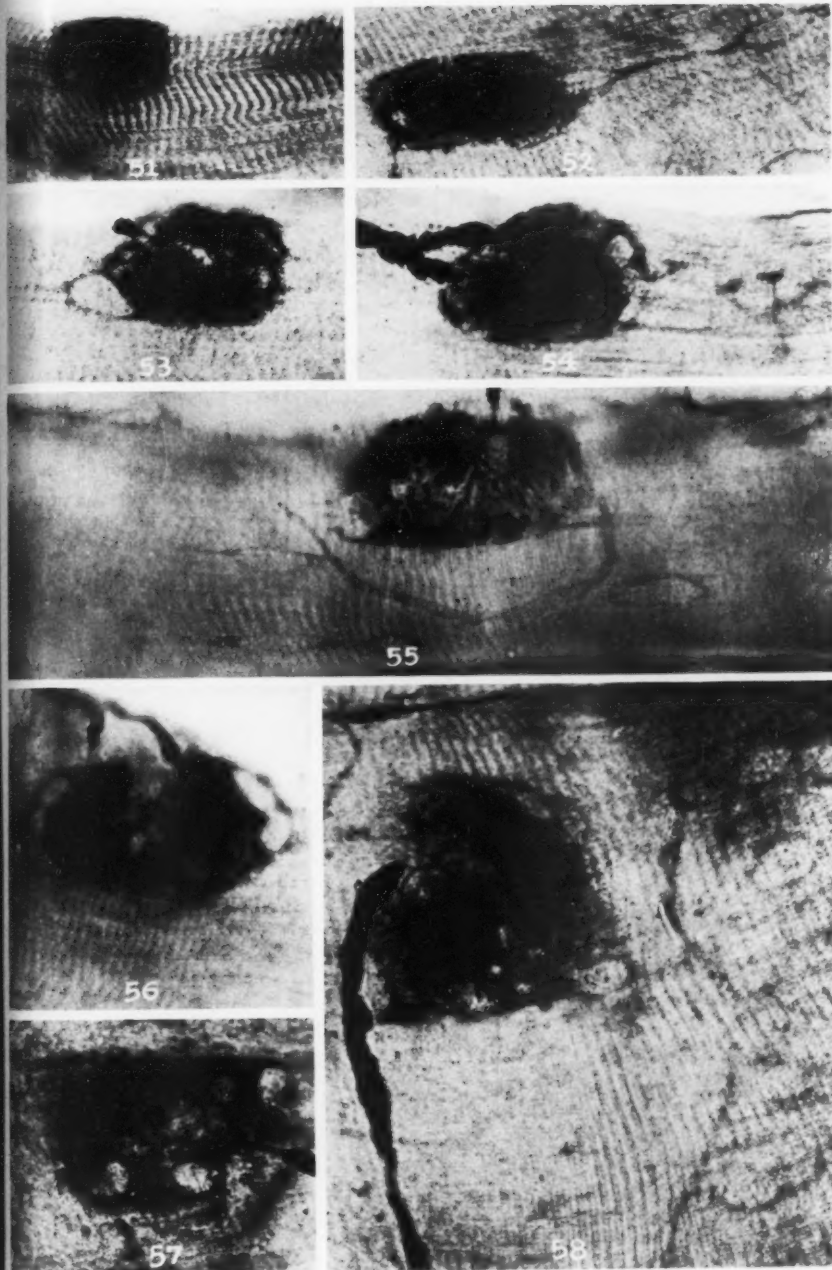minate in end rings, nets, spherules, or as finger-like terminals.

Explosive Ameboid Motion in Nerve Plates

PLATE 49

FIGS. 51 to 58. Pleomorphism of the motor nerve plates produced by superfunctional ameboidism of the axonic terminals in teased right intercostal muscle fibers; hyperpnea due to CO_2 stimulation. The retracted motor nerve plate (Fig. 51) has 13 dark striations in relation with its granular sole plate of Kühne. The partially expanded nerve plate (Fig. 55) has 45 dark cross striations in relation with its remains of the granular sole plate and the axonic motor nerve plate. The single nucleated oval clear area is in a direct stream-like continuity with the nucleated ovoid clear area in the granular sole plate of Kühne (Fig. 52). This stream-line of granules and of irregular granulation of the sarcoplasm is clearly evident (Figs. 47, 53, 54 and 58). The vascular ring around the motor nerve plate, *confluens vasculorum* of Wilkinson, is evident. There are fine oval and fusiform nucleated clear areas in the sarcoplasm scattered at a distance from the motor nerve plate. The irregular scattering of the oval clear areas is likewise shown (Fig. 58). These oval, clear areas, containing nuclei, are scattered out in the muscle substance. They occur in great frequency in practically all muscle fibers, the motor end-plates of which have been greatly expanded by CO_2 stimulation. There is a massive transmission of nerve substance into the axonal divisions in the expanded motor nerve plates, stimulated by CO_2.

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Explosive Ameboid Motion in Nerve Plates

PLATE 50

FIGS. 59 to 63. Pleomorphism of the motor nerve plates produced by superfunc-
tional ameboidism in response to a lethal amount of electrical stimulation in
the right intercostal muscle fibers. The retracted motor nerve plate with coarse
fronds (Fig. 59) has 14 broad, dark cross striations in close relation to it,
whereas the expanded nerve plate (Fig. 63) has 31 fine, dark cross striations
in close relation. There is a clear halo-like zone between the coarse fronds of
the retracted axonic bulb (Fig. 59) and the dark, granular sole plate of Kühne.
The ultra-terminal collateral is observed (Fig. 62) as a fine, non-medullated
branch of the axonal ramification in the typical expanded somatic motor nerve
plate and ends in the same muscle fiber a short distance to the left from the
plate of origin. There is a terminal collateral which arises from the axon a
short distance before it penetrates the sarcolemma and ends as an independent
small plate above the main nerve plate (Fig. 61). There is a replacement of
the regular striations by an irregular granulation. Nucleated ovoid clear spaces,
like vacuoles, occur around the periphery of the motor nerve plate and are
projected along a granular stream-line in the muscle substance to the right of
the nerve plate (Fig. 63). The unipolar expansion of the motor nerve plate
toward the left (Fig. 60) results in fine striae and irregular granulation of
muscle substance toward the left in the direction of nerve plate expansion.
Toward the right the striae are coarse and irregularly placed. The retraction
of the pseudopodia of axonic divisions toward the base of the stem forms a
wide terminal bulb like a growth cone. The lobes of the retracted bulb are
short and wide radicals. There is a massive transmission of nerve substance
into the axonal ramifications in the expanded motor nerve plates, stimulated
by electricity.

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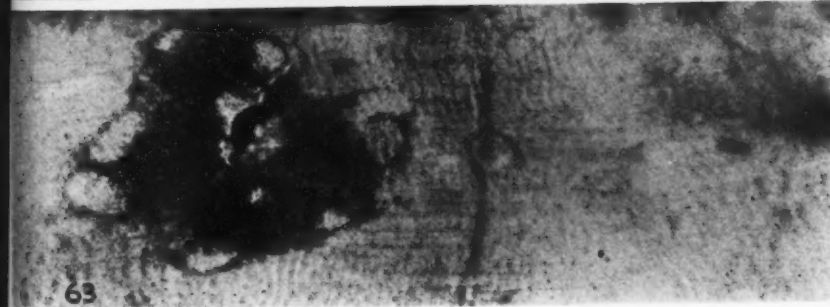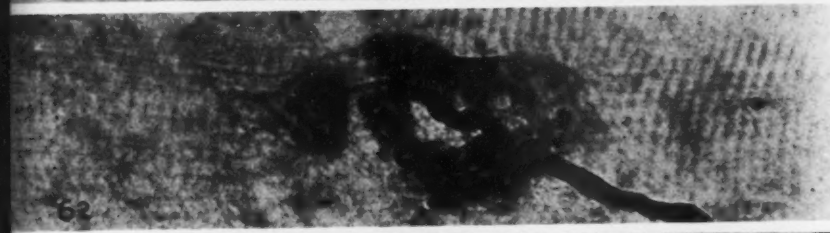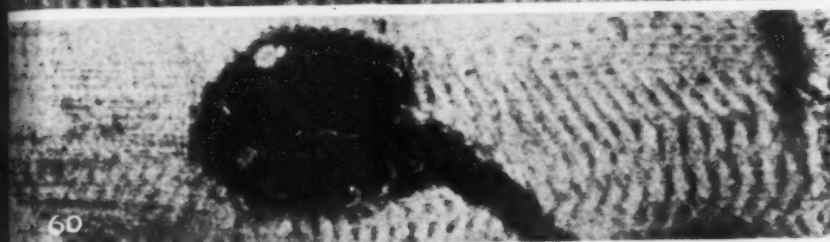
Explosive Ameboid Motion in Nerve Plates

PLATE 51

FIGS. 64 to 67. Pleomorphism by superfunctional and explosive ameboidism; teased right intercostal muscle, stimulated by a lethal alternating current, 120 volts, 1 ampere, for 1 minute. The expanded motor nerve plates have variable relationships to the muscle structure. There is a partial obliteration by a granular streamlining of the cross striations radiating with their convexities to the right of the muscle fiber (Fig. 64). There are fine closely spaced striae related to the kidney-shaped nerve plate (Fig. 65), and an obliteration of the striae related to the nerve plate (Fig. 66) which is supplied by two branches after the single axon undergoes dichotomous division. There is a complete explosive disruption, dissolution, and granular replacement of the arborizations of the axons in the two neighboring motor nerve plates (Fig. 67). This condition is found in 0.5 per cent of the motor nerve ends. There is likewise a replacement of the periodic pattern of muscle striae in this area of sudden and explosive disappearance of the two motor nerve plates by granular disorganization through strong electric stimulation. The nucleated ovoid clear areas are irregularly scattered in the myoplasm surrounding the ghostlike outline of the motor nerve plates. There is a massive transportation of nerve substance into the axonal branches in the expanded motor nerve plates, stimulated by electricity. There is an accompanying perturbation of the related protoplasm of the striped muscle fiber. The surface area of the strongly stimulated motor nerve plate is greatly increased over that of the resting nerve plate.

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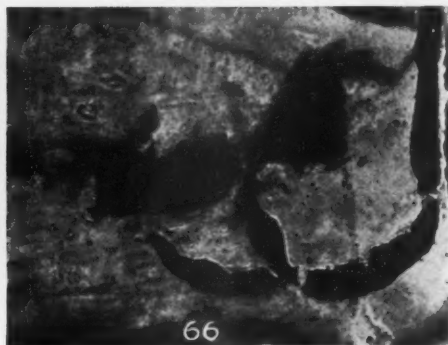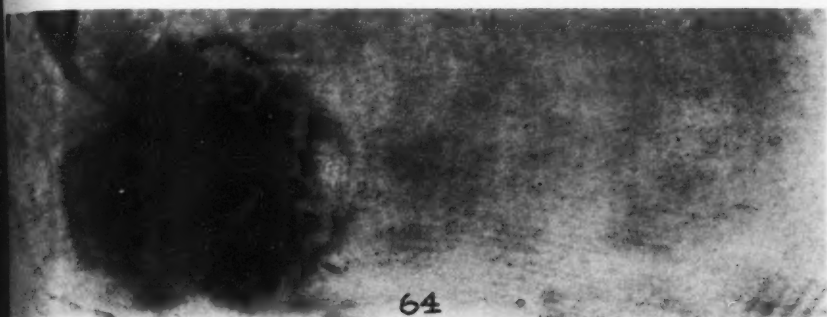
Explosive Ameboid Motion in Nerve Plates

PLATE 52

FIGS. 68 and 69. Photographs of longitudinal sections of rectus abdominis muscle of winter frog, after electrocution by alternating electric current, 120 volts, 1 ampere, for 1 minute, with one electrode in the cervical, the other in the lumbar region. The muscle fibers of the winter frog have central nuclei. The deformations of the relatively passive nuclei, therefore, in the myoplasmic nodes and internodes of electric contracture are definitely portrayed. The nodes are condensed with fine, closely spaced cross striations. The internodes are rarefied with coarse, widely spaced striae or in some places with complete absence of striae. The correlative changes of the nuclei are clearly evident. They are rounded ovoid and compressed in the nodes of condensation; and are elongated or stretched in the internodes of rarefaction. The nodes and internodes of strong contracture are alternately spaced within the single muscle fiber. The myoplasm is more definitely fibrillated in the internodes. These zones of condensation and rarefaction of contracture of the muscle fiber are assumed to be the two components of the longitudinal compression wave which accompanies both the normal and abnormal changes in capillary chemistry in the microcapillary muscle fiber. Hematoxylin and erythrosin stain. × 750.

PLATE 52
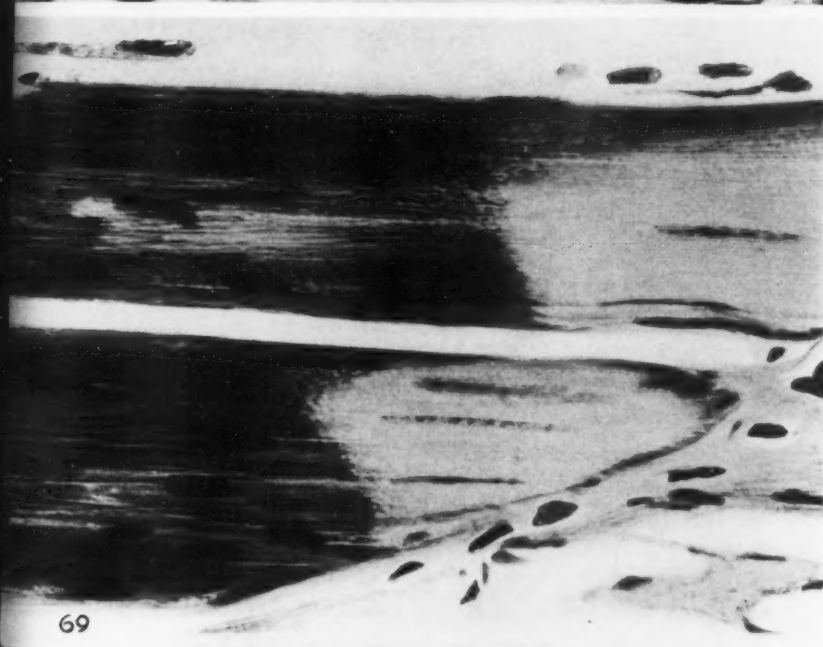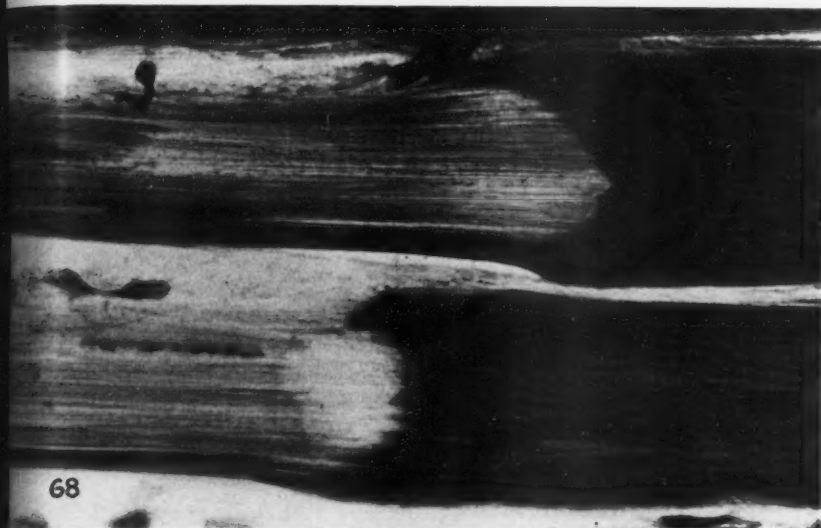
Explosive Ameboid Motion in Nerve Plates
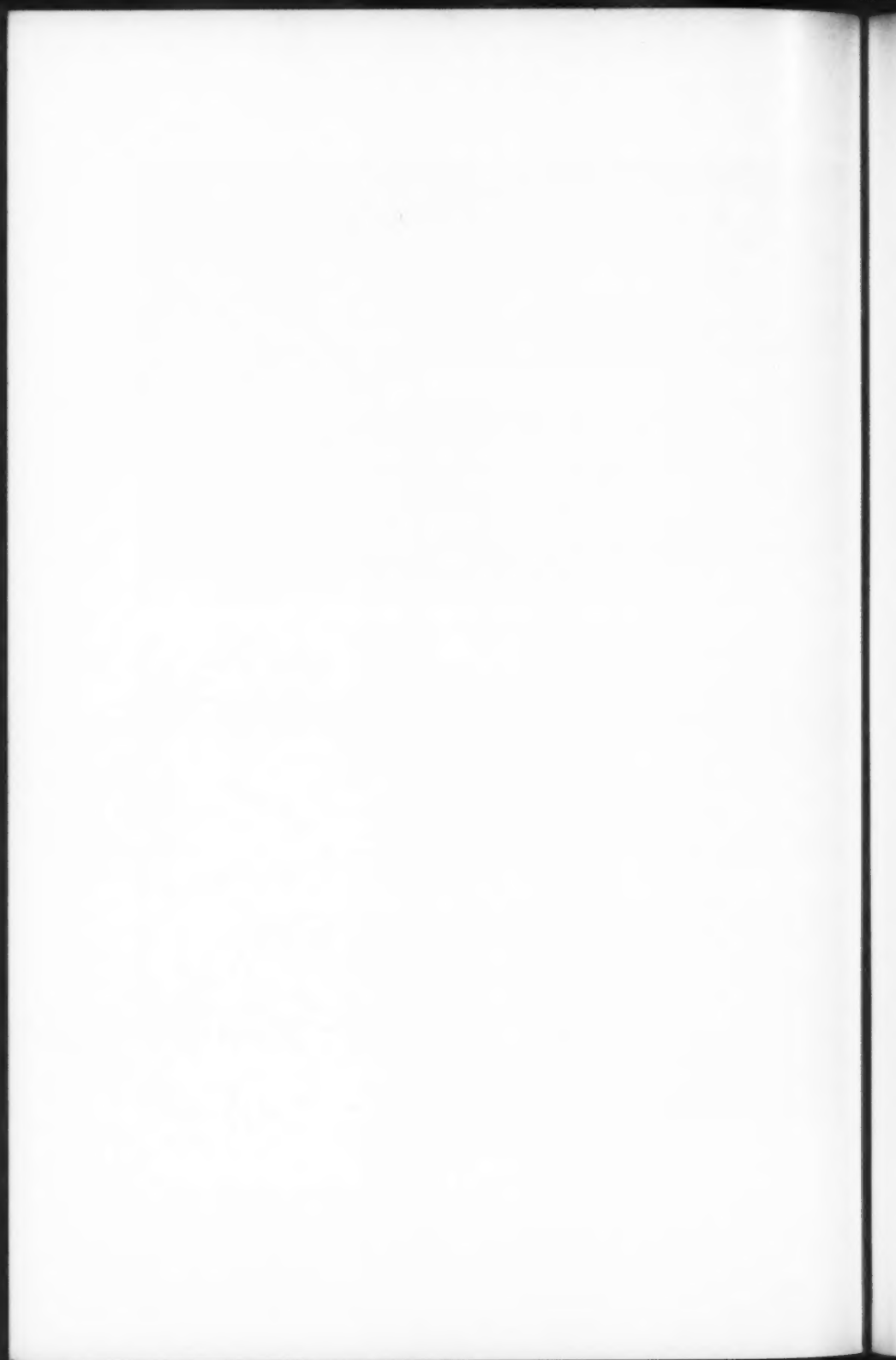
INFLUENCE OF COLCHICINE DURING METHYLCHOLANTHRENE EPIDERMAL CARCINOGENESIS IN MICE*

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A marked reduction in intranuclear viscosity during experimental epidermal carcinogenesis in mice was reported in an earlier paper (Cowdry and Paletta,[1] 1941). It was assumed that when two tissues are subjected to equal ultracentrifugal force, the intranuclear viscosity is less in the one in which the displacement of nucleoli and basophilic chromatin is the greater. Using this material for untreated controls, it seemed desirable to ascertain the influence of colchicine on displaceability in similar tissues, paying particular attention to nuclei in mitosis.

The available evidence that colchicine may have some influence on the viscosity of dividing cells is difficult to evaluate. That alterations in viscosity do occur during mitosis in cells not under the influence of colchicine has been known for a long time. The early literature on the subject has been well summarized by Wilson[2] (1925). It deals, however, chiefly with the eggs of invertebrates, mostly sea urchins, and it is not safe to conclude that similar changes take place in the mammalian cells with which we are concerned, for in their case the stratification of contents requires much greater centrifugal force. But it may be significant for us that Heilbrunn[3] (1920) has stated that in sea urchin eggs (*Arbacia*) the appearance of the spindle depends absolutely on a preliminary cytoplasmic gelation and that suppression of gelation results in every case in suppression of the spindle. The point is that Ludford[4] (1936), on the basis of extensive experience with colchicine, has expressed the opinion that this alkaloid brings about some alteration in the physical state of the mammalian cells which he studied and further that it may prevent spindle formation. If Heilbrunn's conclusion can be extended to include mammalian cells, it would be logical to expect suppression of spindle formation by colchicine in them to be occasioned by failure in gelation, or, expressed differently, by an inhibition of expected increase in viscosity, because Wilbur[5] (1940) has found in *Arbacia* eggs that the increase in viscosity which normally follows fertilization is inhibited by colchicine in a concentration of 1 part to 2000.

Experiments

The mice used in these experiments each received a single subcu-
taneous injection of 0.01 per cent colchicine, the amount being 0.25 cc.
per 20 gm. of body weight. The animals included late embryos and
adults, treated with methylcholanthrene as previously described (Cow-
dry and Paletta,[1] 1941). Care was taken to subject all of the tissues
to the same ultracentrifugal force and to employ the same routine
histological technic.

The material is summarized in Table I. In the first column are
given the nature and the source of the material. The periods of treat-
ment with methylcholanthrene are given in the second column and the

Table I
Experiments

Tissue	Period of methylcholanthrene treatment in days	Hours after colchicine injection	Strains of mice
Embryonic epidermis			
Back			
9 mm. embryo	0	5	Swiss
11 mm. embryo	0	5	Swiss
18 mm. embryo	0	4	New Buffalo
20 mm. embryo	0	5	Swiss
24 mm. embryo	0	5	Swiss
Hyperplastic epidermis			
Back	22	4	Strain A
Back	22	4	Strain A
Ear	55	4	Strain A
Back	75	5	Swiss
Back	75	5	Swiss
Squamous-celled carcinoma			
Back	71	1, 2, 3, 4, 5, 6, 7	Strain A
Back	71	4, 70	Strain A
Back	82	3	New Buffalo
Back	84	0, 5, 26	New Buffalo
Back	98	0, 5, 48	New Buffalo
Back	99	0, 5, 43	New Buffalo
Back	99	0, 5, 43	New Buffalo
Back	110	1	New Buffalo
Back	110	3	New Buffalo
Back	110	6	New Buffalo
Back	110	8	New Buffalo
Back	110	0, 5, 48	New Buffalo
Back	130	1	New Buffalo
Back	130	3	New Buffalo
Back	130	7	New Buffalo
Back	130	4, 24	New Buffalo
Back	130	4, 24	Swiss
Back	140	1	New Buffalo
Back	140	5	New Buffalo
Back	147	1, 24	Swiss
Back	147	2, 24	Swiss
Back	147	6, 24	Swiss
Back	150	2	Swiss
Back	150	10	Swiss
Back	161	4	New Buffalo

hours after the injection of colchicine in the third. From a squamous-celled carcinoma of 71 days, seven biopsy specimens were taken at hourly intervals. From some, specimens were excised before the injection of colchicine, at o hours, but this interfered with the blood supply and the subsequent distribution of colchicine to the tissue so that other specimens collected later on from the same tumor did not show uniformity in colchicine effect.

In addition to the routine technic of Bouin fixation and hematoxylin and eosin staining, other methods were employed in special cases: (1) the Feulgen reaction for thymonucleic acid (Cowdry,[6] 1928); (2) fixation in Regaud's fluid, mordanting in potassium bichromate solution and staining with aniline fuchsin and methyl green for mitochondria; (3) micro-incineration for mineral constituents (Scott,[7] 1937).

OBSERVATIONS

Embryonic Hair Follicles

The change produced by colchicine is more conveniently studied in the hair follicles than in the epidermis itself because of the larger number of mitoses in the former.

Figure 1 shows the epidermis in the upper portion of the field and below a portion of a hair follicle from an embryo, of which the mother received a subcutaneous injection of colchicine 5 hours before its removal and preservation. Five arrested mitoses are included. They are all prophases or early metaphases but in other parts of the specimen a few anaphases and telophases were found. The uniformity of the clumping of the chromosomes is remarkable. The density of the clumps is less than in squamous-celled carcinoma. Comparison of Figure 1 with Figures 7, 8, 11 and 12 illustrating carcinoma cells 5 hours after colchicine injection shows that in the latter the chromosome clumps are without any visible internal organization, whereas in Figure 1 they are less black, and paler areas can be seen within them, indicative of the inclusion of nonchromosomal material (ground substance) and therefore of less dense packing of chromosomes. Moreover, the outlines of the chromosome clumps are definitely smoother in the case of the carcinomatous mitoses, likewise suggesting a closer packing or agglutination. The slightly granular cytoplasm of the embryonic mitoses stains faintly and is small in amount owing to the high nucleo-cytoplasmic ratio.

A portion of epidermis, adjacent to that represented in Figure 1, is illustrated in Figure 2 as it appeared after centrifugation. In the hair follicle in the lower part of the figure, two chromosome clumps have been displaced centrifugally and the areas of cytoplasm, which they originally occupied, have been left behind and appear clear. Small

amounts of stainable cytoplasm can be distinguished. This shifting of chromosome clumps is quite uniform. Their displacement is slightly greater than in control, uncolchicinized tissues but the difference is not easily measured quantitatively.

Hyperplastic Hair Follicles

Figure 3 shows many mitoses arrested by colchicine. The degree of clumping of chromosomes into masses is fairly uniform. The masses are denser than in colchicinized embryonic hair follicles. They appear solid black in the photomicrograph and are therefore more compact than those in Figure 1. Moreover, their outlines are smoother and the ratio of the volume of clump to volume of cytoplasm is less than in Figure 1. This is interesting because, judging from the figures, the nucleocytoplasmic ratio in nondividing cells is not noticeably greater than in the embryonic follicle. Either the cytoplasm of each of the cells in arrested mitosis in Figure 3 took up water or its apparent area is increased by a closer packing of the chromosomes into smaller masses. The fact that the cytoplasm seems clearer in Figure 3 may indicate a difference from Figure 1 or it may be a photographic artefact.

On centrifugation the chromosome masses of colchicinized hyperplastic hair follicles are slightly displaced (Fig. 4); but the displacement is neither so great nor so uniform as in the colchicinized embryonic follicles (Fig. 2). The clumps are larger and less dense than those in colchicinized hyperplastic follicles (Fig. 3). In control, hyperplastic follicles not subjected to colchicine, the displaceability of the chromosome clumps was about the same as in the colchicinized ones. The stainable cytoplasm is slightly displaced (Fig. 4). The chromosome clumps in the few arrested mitoses encountered in the hyperplastic epidermis itself showed much less displacement than those in the hyperplastic hair follicles.

Squamous Cell Carcinomata

The first evidence of colchicine influence was observed 1 hour after injection of the alkaloid, though it may have occurred earlier. It reached a maximum between 4 and 5 hours, was maintained for about 12 hours and subsided in the interval between 12 and 24 hours. The number of arrested mitoses depends upon the rapidity of growth of the tumor and the adequacy of the blood supply bringing in the colchicine. The time from beginning to end of a normal (untreated) epidermal mitosis is not known.

Figures 5 and 6 illustrate early (3 hours) and late (12 hours) stages in the response. In both of the specimens, however, there was

variability and it is not to be supposed that all of the arrested mitoses are like those included in the photomicrographs. These two figures are presented to illustrate some features in the structure of the chromosome clumps and of the clear areas about them in colchicinized carcinomata which have not been centrifuged.

Two mitoses are shown in Figure 5. In the upper one, not fully contained in the section, individual chromosomes can be distinguished but they look slightly swollen and appear to stick together. They are surrounded by thin halos of lightly staining cytoplasm. In the lower, the chromosomes are so closely agglutinated that in the center of the mass no structural details can be made out. The periphery of the mass appears crenated and it is possible that some of the bulgings may represent the surfaces of chromosomes. About the mass there is a more extensive clear area.

Figure 6 shows an arrested mitosis of a large edematous cell. The outlines of the chromosome clump are almost spherical and its surface smooth. The agglutination of chromosomes has progressed farther than in the lower mitosis of Figure 5. The clear area is very much larger and more sharply separated from its environment of rarefied cytoplasm. We shall now describe in a more detailed way the chromosome clumps, clear areas and surrounding cytoplasm.

CHROMOSOME CLUMPS

That the majority of these large, single chromosome clumps are contained in mitoses which have not progressed beyond the metaphase is clear. It is, however, more difficult in colchicinized methylcholanthrene carcinomata to state whether the clumps represent arrests in the prophase or in the metaphase than in hyperplastic or embryonic hair follicles because of the greater density of the chromosome clumps. Often the chromosomes are so close together that longitudinal splitting, if it occurred, would be obscured and equatorial plate formation, if it took place, would be lost by the clumps taking the shape of rough spheres.

A conspicuous feature of the chromosome clumps of late prophases or metaphases is that some of them can be dislodged by the sweep of the knife in the cutting of sections. Figure 7 shows part of a section of a centrifuged squamous-celled carcinoma 140 days after beginning treatment of the epidermis with methylcholanthrene and 5 hours after the injection of colchicine. Two mitoses are included. In the one situated on the right, about halfway between the upper and lower margins of the figure, the chromosome mass appears black, is circular in cross section and is somewhat smaller than the neighboring nuclei. The mass is displaced centrifugally (downward in the figure) so that it

comes in contact with the cell membrane. The basophilic chromatin in the non-dividing nuclei is displaced in the same direction. The one on the left is at about the junction of the upper and middle thirds of the figure. As in the first, the chromosome mass appears solid black; but it has been forced part or all of the way out of the cell in which the space which it formerly occupied can be seen. A wavy line in the cytoplasm may represent part of the nuclear membrane left behind. Such differences in degree of displacement are not unusual.

Figure 8 presents a section from the same block of tissue as Figure 7, but cut in the reverse direction. The sweep of the knife was upward (in the figure) against the direction of centrifugal force. In the upper right a chromosome mass is recognizable by its solid black appearance and slightly crenated margin. It has been carried by the knife out of the position it formerly occupied and which now appears empty. The mass has been pressed against the cell membrane but has not lost its shape. In the three nuclei above it the basophilic chromatin has been shifted in the opposite direction (centrifugally).

In rare cases chromosome masses have been carried completely out of their cells of origin by the knife; but the great majority of them are not displaced by sectioning. Why some are taken while others are left, we are unable to explain. That both look alike can be seen by comparing a displaced one (right in Fig. 7) with an undisplaced one (lower left part of Fig. 8). Examination of sections of 25 uncolchicinized squamous-celled carcinomata did not bring to light a single instance of dislocation of chromosome masses by the microtome knife. The tightening up of chromosomes into such masses, under the influence of colchicine, is more marked in malignant cells than in embryonic or hyperplastic ones.

Figures 9 and 10 supply a very limited comparison of the effect of centrifugation on untreated and colchicinized carcinomata respectively. In the first the displacement of basophilic chromatin and nucleoli in nondividing cells is much greater than in the second. Two chromosome masses are included in the first, one of them partly hidden by the number 9. The other is above it and is somewhat flattened against the cell membrane. Both are much displaced. A single and larger chromosome mass is shown in Figure 10. This is also displaced and is perhaps a little flattened. But other pairs of comparable cells in carcinomata, chosen because the members of each pair appeared to be similar and had been subjected to as nearly as possible the same centrifugal force, provided apparent examples of the reverse result; that is, of an increase in displaceability under colchicine. Still other pairs seemed to indicate that the colchicine was without influence on displaceability.

Attempts were made to make comparisons with tissue from the same tumor. First, a piece was removed and centrifuged. Then, colchicine was injected into the animal and finally the rest of the tumor was excised and centrifuged in the same way. The results were disappointing. The first excision apparently interfered with the blood supply and prevented the colchicine from adequately affecting the remainder of the tumor. That the comparison of treated and untreated neoplasms must be between areas having about the same blood supply is clear from this experience as well as from the observation of Brues and Jackson [8] (1937) that, 10 hours after injection, colchicine does not act in the deeper part of mouse sarcoma 180.

Comparison of tumors removed at various times after injection of colchicine and immediately centrifuged with similar tumors not colchicinized did not settle the question. Each tumor has a more or less pronounced individuality so that the degree of similarity in any comparison was seldom sufficient. And within each there is confusing regional diversity in mitotic frequency, cellular differentiation and degeneration. At present, therefore, we are unable to state whether the displaceability of chromosome masses in squamous-celled carcinomata by centrifugal force is influenced by colchicinization. We think, however, that if there is any influence on displaceability it is not very definite or uniform.

One would look for an increase in the displaceability of chromosome masses by colchicine because, in squamous-celled carcinomata as well as in embryonic and hyperplastic hair follicles, colchicine increases the density of the clumps of chromosomes. But, by the phrase "increase in density," only a reduction in space between the chromosomes is meant, in consequence of which they become more closely or densely crowded together so that in many cases their individual outlines cannot be resolved.

That the clump stains more homogeneously is not sufficient evidence of greater specific gravity. As already stated, some chromosomes in colchicinized tissues are noticeably swollen. If many of them swell, their total volume may be greater even though the area occupied may be less by reason of the smaller amount of ground substance included in the mass.

If the swelling is due to an increase in substance of the same kind, the specific gravity would remain the same. In an earlier paper we found a progressive increase in nuclear and cytoplasmic volumes during carcinogenesis (Cowdry and Paletta,[9] 1941). There is more chromatin to clump. Whether the number of chromosomes in each mitosis has been increased or not will have little effect on the density unless

they undergo some chemical change. If, on the other hand, the swelling is occasioned by intake of water—a possibility indicated by the fact that the centers of the swollen chromosomes sometimes stain less intensely than their peripheral parts—a decrease in specific gravity might be brought about.

The fact that some chromosome masses in colchicinized tissues offer more resistance to the edge of the microtome knife than the aggregates do in uncolchicinized tissues suggests greater density, in the sense explained above, in their fixed, dehydrated, cleared and paraffin-embedded state but not necessarily greater specific gravity *in vivo*. The Feulgen reaction for thymonucleic acid is often slightly different after colchicinization. The chromosome clumps assume a bright cherry red color in contrast with the usual purple color.

After micro-incineration the mineral residues of chromosome clumps in mitoses arrested by colchicine can be identified and in many cases can be distinguished from those of the less dense clumps in uncolchicinized carcinomata. Viewed in the dark field, there is a greater range in size in the light-reflecting granules of the former. The proportion of bright white granules of Ca and/or Mg seems to be increased and that of fine bluish white granules of Na and/or K decreased. Whether there is a change in the red granules of Fe could not be ascertained. Neither could it be discovered whether the total amount of ash of the clumps revealed by this method is modified by the colchicine. But, after colchicine, the ash appeared to be somewhat less well organized in space; in other words, it was not so regular. However, the comparison was only visual and it is particularly difficult in micro-incinerated specimens to so arrange the experiment that the areas of tissue compared are similar. If the comparisons had embraced a large number of better selected tissues, cases might have been found in which the differences referred to were absent.

CLEAR AREAS

Since no adequate search was made for clear areas about the chromosome clumps in living epidermal cells, conclusions as to their nature must be very tentative.

The small halos about individual chromosomes (Fig. 5) may be optical artefacts caused by the refraction of light from surfaces, but the clear area about the upper chromosome mass in Figure 12 is certainly too large to be an optical artefact.

The possibility that the halos are in part artefacts of fixation cannot be altogether excluded because, though the chromosomes are swollen, their size may have been still larger before fixation. In the coagulation caused by the fixative they may have lost fluid, which may have con-

tributed to the formation of the halos. The halos are seen after fixation in the fluids of Bouin, Helly and Regaud; but, of these three, they were most conspicuous after Bouin's fluid.

In centrifuged tissues the clear area is ordinarily of about the same width on all sides of the chromosome clump. Its size seems, in some cases, to bear a relation to the size of the chromosome clump. Thus it is large in Figure 6 and small in Figure 13. But it is not unusual to find clear areas of different sizes about chromosome clumps of approximately the same size (Figs. 12 and 13).

The line of demarcation between the clear area and the surrounding stainable cytoplasm is often distinct (Fig. 6). It may even be membrane-like and stain faintly with eosin or hematoxylin. Thus, the clear area about the lower chromosome clump in Figure 12 appears as if surrounded by a slightly crumpled, nuclear membrane. When specimens like that illustrated in Figure 6 are incinerated, the surface of separation is studded by a mixture of small bluish white and flat white mineral residues suggestive of a nuclear membrane, but the clear area itself is devoid of demonstrable minerals. Another point suggesting, but not proving, that in some instances the line of separation is a nuclear membrane, is the absence of mitochondria within the clear area limited by it, for mitochondria are never within nuclei. When, however, the clear area is small and indistinct, it grades imperceptibly into the cytoplasmic environment and is obviously not limited by a nuclear membrane.

In centrifuged specimens, in which the chromosome clumps have been displaced, the clear areas may wholly encircle them (Fig. 12), partly surround them (Fig. 14) or they may be limited chiefly to their centripetal sides (Figs. 7 and 10), which is inconsistent with their interpretation as artefacts of fixation. Centrifugation does not lead to a suppression of the clear areas by a shifting of stainable cytoplasm into them. The often very sharp margins between clear areas and stainable cytoplasm remain (Fig. 10).

The evidence at hand seems to indicate, therefore, that, when influenced by colchicine, the chromosomes clump together in a dense mass. They withdraw themselves to some extent from the surrounding substance and leave a clear area which is intranuclear and more conspicuous in those cases in which a nuclear membrane persists even as a vestige.

SURROUNDING CYTOPLASM

The stainable cytoplasm of colchicinized cancer cells is not always of the same density. Its appearance depends somewhat upon the fixation but there may be a definite hydropic change. The displaceability

of the stainable cytoplasm frequently is less when it is more hydropic, or rarefied, as may be seen by comparing Figure 12 with Figure 11. In some cells even the chromosome clumps are not displaced (Fig. 13). Particularly is this the case in those whose cytoplasm is strongly acidophilic when stained by hematoxylin and eosin. The affinity for eosin of cytoplasm in intestinal cells of mice 8 hours after the injection of colchicine was noted by Clearkin [10] (1937). Centrosomes and spindle fibers were not observed by us, but a special search was not made for them in iron hematoxylin preparations appropriately counterstained.

In preparations for the demonstration of mitochondria it is a simple matter to select a number of mitoses which, from the appearance of their chromatin masses, seem to have been equally arrested by the colchicine. But in them the properties of the mitochondria are not uniform. In some the mitochondria have the usual rodlike and filamentous shape while in others they have been changed into deeply staining spherules which may be of variable size and of uneven distribution in the cytoplasm. In still others the mitochondria are represented only by fairly large vesicles with more or less clear centers. There is, in other words, a gradation from mitochondrial contents that appear to be normal, to others indicative of injury and of death, or of approaching death. We are unwilling to commit ourselves as to how far spherule and vesicle formation can proceed while the possibility of reversion to normality remains.

That these differences between the mitochondrial contents of arrested mitoses are not due to variations in technic is evident from the fact that they occur in the same preparation in cells equidistant from the surface of the tissue. There are two other possibilities. The mitochondrial changes are either a primary result of colchicine action or a secondary result in consequence of the mitotic arrest. It is conceivable that the mitochondria are most altered in the mitoses held longest in the metaphase. Perhaps some of the arrested mitoses do not survive and the mitochondria are cytoplasmic indications of serious injury which is not marked by further detectable alterations in the chromosome masses.

DISCUSSION

In the tissues examined and under the conditions specified, colchicinization renders the chromosome masses slightly more displaceable by centrifugal force in embryonic hair follicles but no consistent difference was noted in their displaceability under colchicine in hyperplastic hair follicles and in squamous-celled carcinomata in the methylcholanthrene series.

It is possible that, in the embryonic hair follicles which were not

subjected to methylcholanthrene, the increase in displaceability on col-chicinization was caused by a decrease in viscosity of the ground sub-stance. But the chromosomes in the mitoses arrested by colchicine differed from those not under colchicine. They were more closely packed together. Whether this difference is accompanied by an altera-tion in their specific gravity we do not know; but if their specific gravity is increased relative to that of the ground substance, their ten-dency to displacement would also be increased because the push, or the pull, exercised by the centrifugal force would be greater.

Conditions in the tissues previously treated with methylcholanthrene are more complicated. In them the density of the chromosome clumps is greater than in the embryonic follicles to which the carcinogen was not applied, especially in the carcinomata which were subjected to it over a longer period. Some of the clumps in the carcinomata offered more resistance to the sweep of the microtome knife, were colored a brighter cherry red by the Feulgen reaction and exhibited a different mineral residue on micro-incineration than those in either the hyper-plastic or embryonic follicles. In addition the ground substance in the arrested mitoses differed from that in mitoses not under the influence of colchicine. It was divisible into characteristic clear areas about the chromosome clumps and into investing cytoplasm, the mitochondrial content of which was modified. Consequently, the possibility of changes in specific gravities under colchicine, which might partly condition displaceability by centrifugal force, is considerably enhanced; but there is no evidence that the differences mentioned are actually cor-related with alterations in specific gravity.

If, however, changes in the specific gravities of chromosomes, clear areas and cytoplasm are produced by colchicine, the fact that displace-ability is not significantly altered does not necessarily mean that the colchicine is without influence on viscosity. Obviously, an increase in viscosity of the ground substance of the mitoses might not result in lesser displaceability of the components if their specific gravities were so increased that they became more susceptible to displacement by the centrifugal force. It is equally evident that a decrease in viscosity of the ground substance might not result in greater displaceability if their specific gravities were so decreased that they became less suscep-tible to displacement. Our experiments with the ultracentrifuge there-fore do not indicate either a change or an absence of change in viscosity of the ground substance of mitoses arrested by colchicine in hyper-plastic hair follicles and carcinomata.

It is doubtful whether the larger of the clear areas which we have described about the chromosome clumps are similar to those reported

by Ludford [4] as occurring in Flemming preparations stained with iron hematoxylin and which he thought might represent spindle substance. Evidence is altogether lacking that the clear areas in our specimens are composed of spindle substance. Their chief characteristics are lack of stainability and absence of detectable mineral constituents. Their shape is in no way suggestive of spindles, but they occur near the chromosome masses. This is the normal site of spindle development. A feature of the clear areas is that they tend to hold their shape and do not merge with the stainable cytoplasm on ultracentrifugation. They may be areas of gelation or of increased viscosity; but, if this is the case, the absence or rarity of spindle formation reported by Ludford and others would not be correlated with a failure of gelation—a possibility mentioned in the introduction to this paper.

Heilbrunn [3] has mentioned abstraction of water as one cause of gelation in *Arbacia* eggs. Other things being equal, loss of water would bring about a decrease in volume. Apparently no one has made detailed measurements of normal and colchicinized mitoses to ascertain whether a change in volume actually does take place, but Allen, Smith and Gardner [11] (1937) noted that mitoses under colchicine are of large size and round or oval shape. In our preparations the cells in mitosis are rounded and some (Fig. 6) appear to be swollen. If the arrested cells actually become more watery, it would not be surprising to discover a decrease in viscosity; but a change in viscosity can occur without change in volume by redistribution of water, as when a colloidal solution gels. The possibility that colchicine in some cases facilitates the entry of water into mitoses is interesting from another angle since it may explain why tumors under the influence of colchicine are more susceptible to the destructive action of distilled water, as has been reported by Guyer and Claus [12] (1940).

According to Ludford,[4] the prophase is the phase of mitosis least affected by colchicine because the nuclear membrane is impermeable to it. Our observations of clumped chromosomes in mitoses provided with what looked like nuclear membranes, may not be of significance in this connection for such membranes were seen only in the carcinomata in which the range of variation of the cells was much wider than in the embryonic or hyperplastic hair follicles. Indeed, the nuclear membranes in question may have become so altered that colchicine could get in. Since Scott [13] (1936) has been successful in the analysis for minerals of nuclei separated from cytoplasm and collected in fair quantity, it may be feasible, in a later study, to determine whether colchicine can enter nuclei. Another test would be to ascertain whether colchicine arrests mitoses in those forms, mentioned by Wilson,[2] in

which the nuclear membrane persists throughout the whole process of mitosis.

Like others, we have found some variability in the appearance of arrested mitoses. This effect was most marked in the methylcholanthrene carcinomata, less in the methylcholanthrene hyperplasia and least in the embryonic hair follicles. Despite a certain range of variation in almost any specimen, it is appropriate to recognize two types of arrest. The first is the "condensed arrest," described in this paper and in many others, in which the chromosomes are drawn together or "contracted" into a clump. The second may be called "dispersed arrest." The complete scattering of chromosomes described by Brues and Jackson [8] as a first stage in the reaction of regenerative (compensatory) mitoses in the livers of rats to colchicine comes under this heading. Not only the first stage but, as far as the information goes, the whole process resembles the "forme chromatorhexique" of arrest reported by Dustin and Zylberszac [14] (1939) in compensatory mitoses in the kidney. A similar dispersal of chromosomes and of small chromosome clumps was described by Dustin and Chodkowski [15] (1938) in endothelial cells in colchicinized cicatrizations, but, judging from the figures of some of the arrests, the chromosomes were condensed, not dispersed. Perhaps the two types may exist side by side in the same tissue. Dustin and Zylberszac suggest that the "forme chromatorhexique" is occasioned by strong concentration of the poison in organs like the liver and kidney which eliminate it. But it is unsafe to assume that mitoses in different tissues in normal growth, as well as in compensatory hypertrophy, respond equally to equal concentrations.

Quite apart from the relationship between the two types of arrest, it is evident that colchicine can bring about either a condensation or a dispersal of chromosomes. The physical forces operating would appear to be different in the two cases. Ultracentrifugation and micro-incineration of the dispersed arrests might yield entirely different results from those obtained by us with contracted arrests.

To predict the action of colchicine on any particular tissue from descriptions, however accurate, of its influence on other tissues is to court mistakes. We have found slight differences in its action on epidermis and hair follicles of mice, and Carleton [16] (1939) noted a difference in the response of the skin to colchicine in rats of the same strain and even of the same age, but she did not refer to any difference between sexes. The claim of Havas and Gal [17] (1938) that methylcholanthrene sensitizes a great number of cells to colchicine is neither supported nor contradicted by our experiments, since we made no counts, but the chromosome clumps in the contracted arrests in our

methylcholanthrene-treated tissues were denser than in untreated tissues. Other sensitizers and inhibitors may lurk in the background. The evidence is strong that in some cases colchicine is a mitotic stimulant increasing the number of mitoses (Paff,[18] 1939, and others). It is equally convincing that in other instances colchicine is a mitotic inhibitor decreasing the number of mitoses (Tennant and Liebow,[19] 1940). Failure to find evidence that colchicine acts in either of these two ways is without value except for the particular tissues investigated. For these reasons it seems to us, as to Worthington and Allen [20] (1939), that the validity of using colchicine-arrested mitoses as indices of growth rates depends on proof that the accumulated mitoses are proportionate to those normally occurring in the particular tissue. To prove this point would probably be more complicated and difficult than to estimate growth rates without using colchicine.

SUMMARY

The susceptibility to colchicine of mitoses in embryonic hair follicles, in hair follicles rendered hyperplastic by methylcholanthrene and in squamous-celled carcinomata produced by methylcholanthrene appeared to be slightly different.

In embryonic hair follicles the clumping of chromosomes was less dense than in cancer cells, when both were observed 5 hours after injection of colchicine. The displacement of the clumps by centrifugal force was slightly greater in colchicinized than in uncolchicinized embryonic epidermis.

In hair follicles made hyperplastic by methlycholanthrene, the clumping of chromosomes by colchicine was more marked than in the embryo and less than in cancer cells. The displacement by centrifugal force was less than in embryonic hair follicles and in cancer. No difference was noted between the displacement of the clumps by centrifugal force in colchicinized and uncolchicinized hyperplastic hair follicles.

Squamous-celled carcinomata produced by methylcholanthrene were examined over longer periods of colchicine influence and at more frequent intervals. The chromosomes were more closely packed in clumps than in either the embryonic or the hyperplastic hair follicles. A few of the clumps were dislocated by the sweep of the microtome knife in cutting sections—a phenomenon not observed in 25 uncolchicinized carcinomata in which a search for it was made. Some evidence was found that the thymonucleic acid and mineral contents of the chromosome clumps differ from those of the less compact clumps in uncolchicinized tissues. The chromosome clumps were surrounded by clear areas of variable size. The larger ones were mineral-free in micro-incinerated

sections. The investing cytoplasm varied, as between different mitoses, in its affinity for hematoxylin and eosin and in its mitochondrial content, even when no difference could be distinguished in the chromosome clumps.

The displaceability of the chromosome clumps, clear areas and stainable cytoplasm, under centrifugal force, was less uniform in carcinomata than in the embryonic and hyperplastic hair follicles. No consistent difference in displaceability was observed between colchicinized and uncolchicinized carcinomata. Evidence could not be adduced that colchicine causes a change in the viscosity of mitoses, because alterations in specific gravities may occur, but the possibility that a change in viscosity may take place was not excluded.

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DESCRIPTION OF PLATES

PLATE 53

FIG. 1. Epidermis taken from the back of a 20-day mouse embryo, showing arrested mitoses. Specimen obtained 5 hours following the injection of colchicine subcutaneously into the mother's thigh. × 1040.

FIG. 2. Centrifugation of a small piece of skin adjacent to that shown in Figure 1. Marked centrifugal displacement of the chromosomes and centripetal clearing of the cytoplasm are seen. In all centrifuged specimens the force was about 350,000 times gravity for 30 minutes. × 1040.

FIG. 3. Epidermis of a Swiss mouse rendered hyperplastic by painting with methylcholanthrene for 75 days, showing arrested mitoses in hair follicle. Specimen was biopsied 5 hours following the injection of colchicine. × 1040.

FIG. 4. Centrifuged skin of the back of a mouse showing downward shift of chromosomes in arrested mitoses. Specimen obtained from tissue adjacent to that shown in Figure 3. × 1040.
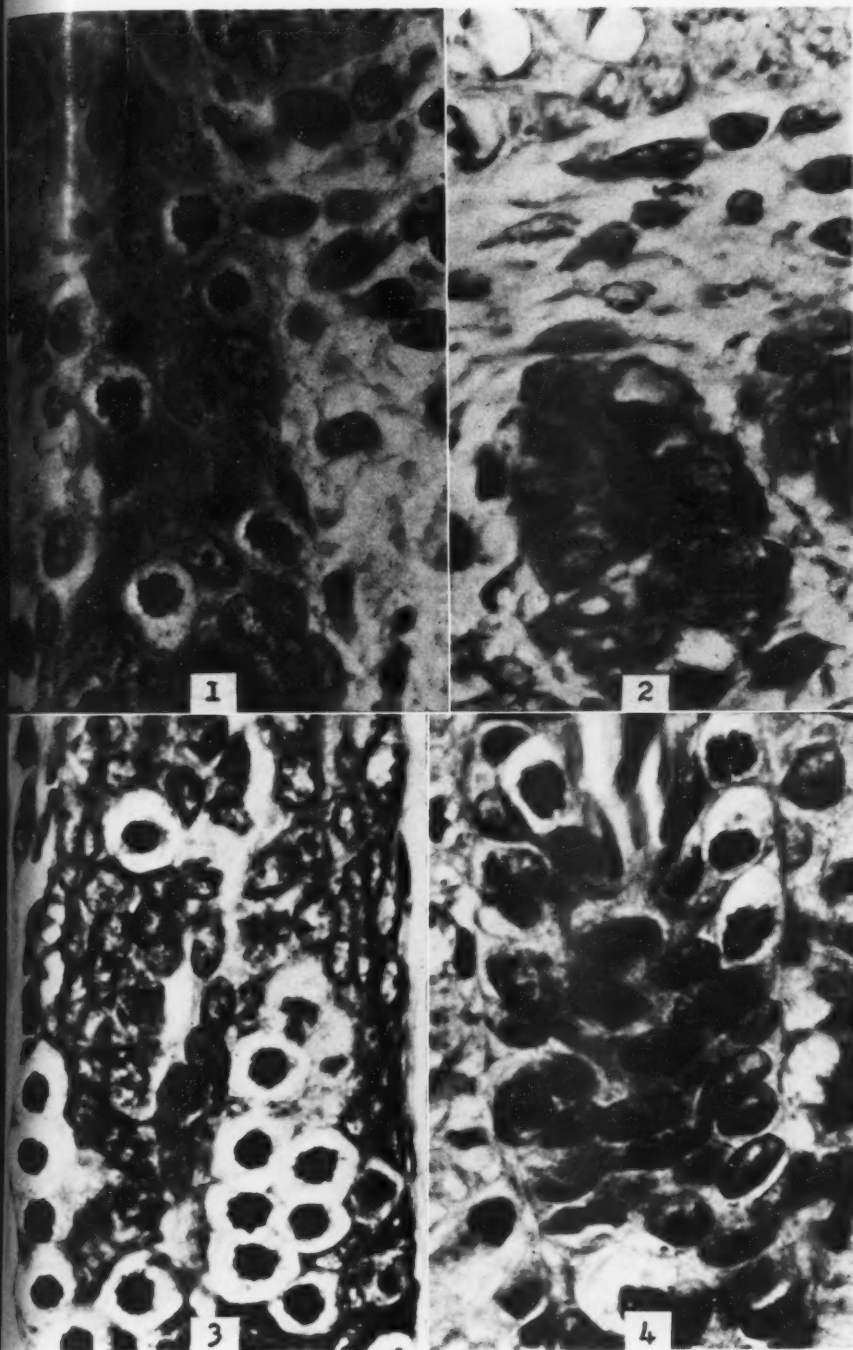
PLATE 54

FIG. 5. Cancer cells arrested in division by the action of colchicine. The upper dividing cells show the fragmented appearance of the chromosomes. The lower cell shows a tightly clumped mass of chromosomes surrounded by a dense cytoplasm. Specimen taken from a mouse injected with colchicine 3 hours previously. (Methylcholanthrene, 130 days.) × 1040.

FIG. 6. Large mitosis arrested by the action of colchicine. A clear zone can be seen surrounding the agglutinated chromosomes and the vacuolated cytoplasm. Taken from the same specimen as Figure 5. × 1040.

FIG. 7. Centrifuged tissue from squamous-celled carcinoma 5 hours following the injection of colchicine. The resting cancer cells show displacement of chromatin and nucleoli centrifugally. Chromosomes are seen which were carried out of the cell in the upper left by the cutting knife. (Methylcholanthrene, 140 days.) × 1040.

FIG. 8. Prepared from the same centrifuged specimen as Figure 7, only the tissue was cut in the opposite direction. Here the chromosomes were forced out of the cell in a direction opposite to that shown in Figure 7. × 1040.
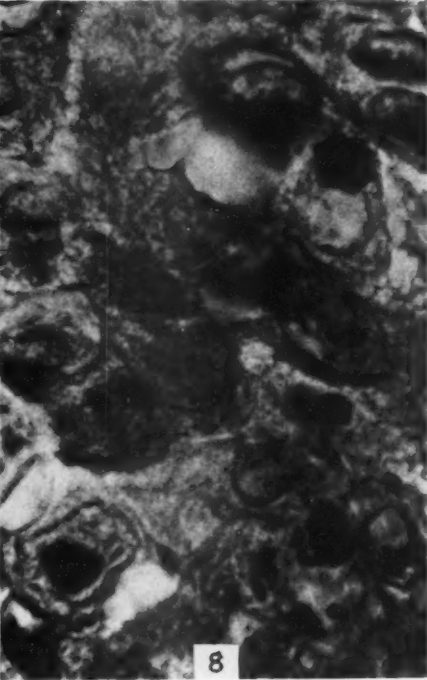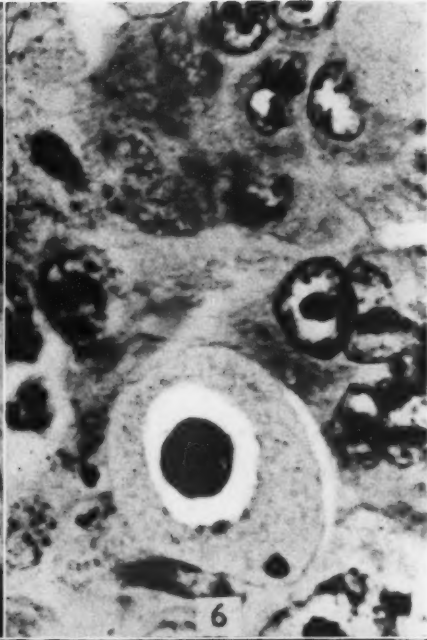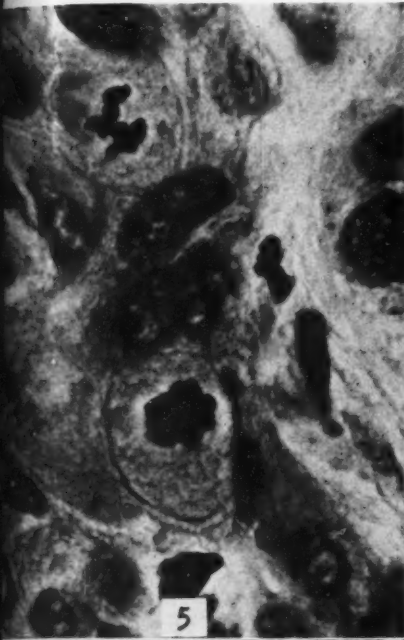
PLATE 55

FIG. 9. Centrifuged tissue of a squamous-celled carcinoma. This mouse was *not* injected with colchicine. The chromosomes and cytoplasm have shifted to the centrifugal side of the cell. Compare with Figures 10 to 14. (Methylcholanthrene, 166 days.) × 1040.

FIG. 10. Centrifuged tissue taken from a squamous-celled carcinoma. This mouse was injected with colchicine 5 hours previously. Although the chromosomes are at the centrifugal side of the cell, the shape of the clear zone and of the displaced cytoplasm behind it has been retained. (Methylcholanthrene, 140 days.) × 1040.

FIGS. 11 and 12. Centrifuged squamous-celled carcinomatous tissue obtained from a mouse which had colchicine injected 5 hours before the biopsy was taken. The shape of the clear area around the chromosome mass has been retained and the shifted cytoplasm does not break through the clear zone. Resting cancer cells show displacement of nucleoli and chromatin centrifugally. (Methylcholanthrene, 140 days.) × 1040.

FIG. 13. Tissue from squamous-celled carcinoma that had been centrifuged after colchicinization for 6 hours. There has been relatively little or no shift of the chromosomes. Compare with Figure 9. (Methylcholanthrene, 147 days.) × 1040.

FIG. 14. Centrifuged squamous-celled carcinoma after colchicinization for 5 hours. (Methylcholanthrene, 140 days.) × 1040.

PLATE 55
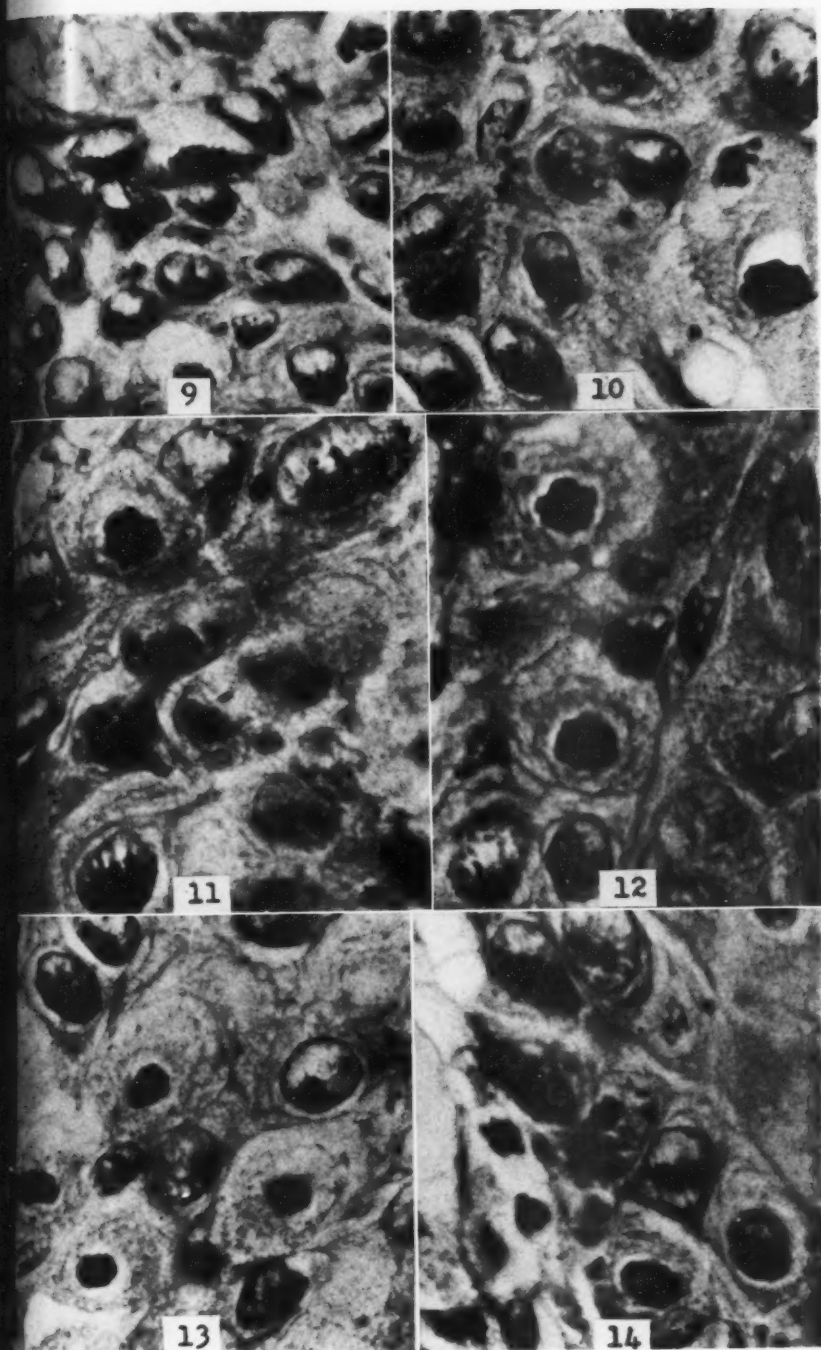
Influence of Colchicine During Carcinogenesis

SILICOSIS OF SYSTEMIC DISTRIBUTION*

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The present conception of silicosis, in the words of the Committee on Pneumoconiosis and the Committee on Standards of the American Public Health Association, at a joint meeting in 1932, is that it "is a disease due to breathing air containing silica (SiO_2) characterized anatomically by generalized fibrotic changes and the development of miliary nodulation in both lungs."

Except for the associated deposit and similar fibrosis in peribronchial lymph nodes, which is an integral part of the lung disease, there has been no report of free silica deposit and associated anatomical changes in other viscera in naturally occurring silicosis.

Gardner and Cummings [1] have, however, demonstrated that by the injection of particulate free silica directly into the circulation in certain animals a state of experimental silicosis may be produced which is comparable in tissue damage and fibrosis to naturally occurring silicosis in man.

From a postmortem study, herein recorded, it may be concluded that the possible occurrence of silicosis should be widened to encompass the deposit of silica in such locations as the spleen, liver and kidney, with structural changes in at least the first two of these organs. Whether or not this may also entail consequential anatomical damage, especially in the case of the liver, or the possibility of other systemic effects of a toxic nature, can be no more than speculative at the present.

REPORT OF CASE

The following case study is presented as evidence that silica and silicosis are not necessarily confined to the pulmonary area.

The subject was a negro man, 30 years of age, who worked in a sand-drying plant "the majority of his time" for the 6 years prior to his illness. The information came from his physician, who provided the only account of this patient's exposure to dust and of his illness. It was said that in his work he was exposed to very dusty conditions, with no protective measures available, in that he was subjected to the inhalation of fine sand in quantity, blown by heated air.

He was first seen by a physician in March, 1937, and was treated for a fever, judged to be malaria, after which he returned to work for a few days. In April he was seen again, when he complained of fever and general bodily aching. His temperature was then 100° F., his pulse rate was 100 and physical examination revealed nothing but "occasional inconstant râles" at the bases of both lungs. Malarial para-

sites were found in the blood, and after antimalarial treatment he returned to work for about 1 week. When seen again in June his condition was apparently the same, save that he stated that he had been coughing considerably and had apparently lost some weight. His temperature was then "slightly elevated" as was his pulse, and râles over both sides of the chest were constant. X-ray examination of the chest at that time was thought to show tuberculosis and he was put on appropriate treatment. The roentgenogram of the chest, seen by me after the autopsy, exhibited rather heavy but irregular shadows in the mid three-fourths or more of the lungs, appearing to follow bronchial markings generally. The apices and bases were comparatively clear. No definite nodules were seen. There was some obscurity laterally near the right apex. The diaphragm appeared high on both sides. The heart borders were obscured by lung densities. When the patient was seen again in November it was said that he gave the impression of an advanced case of tuberculosis, with râles over the entire lung area and friction rubs over both lungs anteriorly and posteriorly. Two months before he died there were signs of complete collapse of the left lung, with friction rubs and râles over the entire right lung area.

Since this man was not under close observation and conditions for thorough clinical study were not available, the clinical record of the case is inadequate. He died in March, 1938, about 1 year after he first became ill, and I made a postmortem examination about 24 hours later under conditions offering minimal facilities. The autopsy protocol (no. 46134–38–56) follows.

Gross Examination

The body was that of a male Negro of rather small stature, quite emaciated, apparently 20 to 30 years of age, in a state of good preservation, having been dead about 24 hours. There were no superficial abnormalities except that the fingernails were bluish, and there was very little fatty tissue. The fingers and toes showed no clubbing.

The right lung was collapsed so far as was possible because of adhesions and solidification. The pleural cavity contained air, the surfaces were shiny and there were a number of heavy pleural adhesions, particularly in the midregion both laterally and posteriorly. The base was free and there was only a small amount of clear brown fluid in the cavity. This lung was comparatively small and in a solidified, contracted state except at the margins and the apex, where, as well as occasionally over the solidified portion, there were air-bearing areas with large emphysematous blebs. The lung sectioned with distinct resistance, being fibrous and apparently gritty. The middle three-fourths, or more, was of a densely solid, dark gray mottled color, the surface being, in general, depressed, with some particularly retracted scars. At the apex the lung tissue was not solid but fibrous strands appeared in it. There were no cavities and no areas of recognizable necrosis.

The peribronchial nodes were enlarged, some to the size of a kidney bean, and had a dark gray marbled appearance.

The left pleural sac had a reddened rough surface, with some deposit

of fibrin upon it, and there were fewer fibrous adhesions than on the right, these being in the midportion and toward the apex but not at it, and laterally and posteriorly. There was about a teacupful of brown flocculent fluid in this cavity. The left lung was very much like the right in the solidified, resistant, gritty, dark gray marbled, densely solid state of the mid three-fourths or more, with depression of the surface and contracted scars in the solid area. There was also air-bearing tissue with large emphysematous bullae in the apex and margins. No areas of necrosis or cavitation were found.

The peribronchial lymph nodes were in a dark gray dense state similar to that of the nodes on the right.

The heart was enlarged laterally, the enlargement being due to hypertrophy of the right ventricle and to right-sided dilatation. The left ventricle, auricle, valves and vessels and the aorta were of normal appearance and the chambers were filled with currant jelly clot which was beginning to soften.

The liver, spleen, kidneys, pancreas, gastro-intestinal tract and other abdominal organs and structures were of normal appearance and position to superficial examination.

Microscopic Examination

Lung. The pleura was thickened by fibrillar and partly hyalinized connective tissue throughout, that of the left lung being covered by a layer of fibrin containing a few leukocytes. Sections from the right lung showed variable degrees of collapse of the vesicles in the relatively non-fibrosed portions. In the apex of the right lung some small vessels showed thrombosis and there was fresh hemorrhage, probably related to a rupture which caused the pneumothorax.

In the apices, with areas of emphysema, there were a number of rounded, laminated, hyaline nodules containing deposits of fine, granular dustlike particles. These nodules were in part separate but also occurred in groups. In addition there were deposits of black dust in a perivascular distribution. The alveoli in some areas showed an accumulation of edema fluid, and in this also there were scattered fine dust particles.

Sections from the lower parts of the lungs revealed a condition similar to that found in the apices, but greatly aggravated. Numerous single and grouped hyaline, laminated nodules, in which occurred deposits of fine dust particles, occupied a large portion of the lung space. In the periphery of these nodules, and between them when they were grouped, there was proliferation of young fibrous tissue. There was variable emphysema and collapse and in many open alveoli edema fluid discol-

ored by fine dust particles was encountered. In some sections practically all of the lung tissue was obliterated by masses of hyaline nodules, with intervening cellular fibrous tissue.

The bronchi in the dense fibrous areas were in a state of partial to complete collapse and the mucosa of the large bronchi showed epithelial metaplasia.

Liver. In addition to central lobular congestion and associated deposit of brown, granular, iron-bearing pigment, there were deposits of fine granular, opaque dustlike material, located also about the central lobular venules and in the adjacent sinusoids. In addition, this material was found in the connective tissue about the collecting veins. It was accumulated particularly in Kupffer cells in the central lobular, swollen zone, giving a grayish cast to these cells under low magnification. It also occurred in hyaline and fibrillar strands of apparently necrotic material in the central lobular zone, among which were scattered nuclear particles. Where focal deposits were heaviest and liver cells were degenerated or necrotic, there were a few leukocytes and also a few large spindle-shaped fibrous tissue cells. While there was no tissue change shown in the portal vein and bile duct, high magnification revealed some fine granular deposit in the surrounding connective tissues.

The picture given by the liver was that of foreign dustlike material which had been taken out of circulation by the Kupffer cells of the central zone and which, where deposited, had led to degeneration and necrosis of tissue, with hyalinization and early fibrous tissue proliferation. Since practically every lobule showed some deposit, the volume appeared to be considerable with a material loss of liver substance.

Spleen. The capsule was not materially altered. The walls of the arterioles of the malpighian corpuscles were usually, though not invariably, thickened and hyaline. The fibrous trabeculae were prominent and the walls of the vessels within them also showed hyaline thickening. The sinuses were generally full of blood and their walls were prominent and cellular, with the endothelial cells conspicuous. Within the walls of the sinuses, and especially in the endothelial cells, there was a deposit of fine, opaque, dustlike granules, so small that examination with the 16 mm. objective and the 10x ocular did not reveal them. Like material was also deposited in broad zones or in more or less rounded collections within the trabeculae, especially in the periphery of these structures, and within some of the malpighian bodies, here lying along the arteriole as a rule. In the trabeculae and malpighian bodies this material lay within more or less hyalinized tissue, which was neither especially rounded nor laminated, although in some instances there

was a suggestion of nodularity. Within this hyaline matrix were numerous, large, ovoid or spindle-shaped cells, more numerous at the periphery and fewer at the center of the malpighian body. Scattered about were nuclear fragments from necrotic cells. These also were more numerous in the periphery of the body.

The picture as a whole is that of deposit of very fine, opaque granular dust, in the endothelial cells of the sinuses and in nodular or band-like collections, stimulating connective tissue formation, hyalinization and even inducing necrosis, within malpighian bodies and the peripheral zone of the trabeculae.

Kidneys. Cursory examination of the kidneys showed no particular abnormality save congestion and cellular degeneration, attributable to postmortem autolysis. Closer study, however, revealed the presence of very fine opaque granules scattered in the endothelial cells of the capillaries of the glomeruli. There was no apparent change associated with their presence, unless there may have been some hyalinization of the basement membrane.

Peribronchial Lymph Nodes. The lymphoid tissue of the nodes adjacent to the large bronchi was, to a large extent, replaced by masses of hyaline nodules of various sizes, containing fine deposits of particulate matter. The centers of some of the larger nodules were calcified.

In none of the organs was there found any evidence of tuberculosis, and no acid-fast bacilli could be found in appropriately stained sections of liver and spleen. The Prussian blue test failed to demonstrate iron in the particles in any of the tissues.

The diagnosis at autopsy was: Pulmonary silicosis; acute pleurisy, left; pneumothorax, right; hypertrophy and dilatation of right heart. After microscopic examination, to this diagnosis the following was added: silicosis of spleen, liver, kidneys and peribronchial lymph nodes. Because of conditions surrounding the autopsy and since there appeared to be no abnormality of any organ except lungs and heart, only blocks of other viscera were taken for microscopic study.

Both grossly and histologically the condition of the lungs was typically that of silicosis and the deposits in the spleen and liver appeared to be of the same material. However, since the natural occurrence of silicosis outside the pulmonary area had not been observed, it was important that the material concerned should be identified.

Microchemical methods of analysis* were devised by F. B. Culp, of the Chemistry Department, since the tissue taken from the liver,

* Mr. Culp proposes to publish his methods of these examinations in a separate article.

spleen and kidney was only that which was intended for histologic study. In this chemical examination the lung was found to contain 1.6 mg. of silica to each gram of wet tissue and 11.15 mg. of silica to each gram of dry tissue. By referring to the chemical examination for silica in lungs in health and disease by McNally,[2] this will be found to be a higher content of silica than was found in all but two of his eight workers in dusty atmospheres, and significantly greater than his findings in lungs of persons not so exposed. Control tests on three non-diseased lungs, selected to have had a minimum of dust exposure, gave 0.050, 0.019 and 0.031 mg. of silica to each gram of wet tissue and 0.50, 0.13 and 0.18 mg. to each gram of dry tissue, respectively.

The same analysis applied to liver tissue in the present case revealed 0.10 mg. of silica to each gram of wet tissue and 0.70 mg. to each gram of dry tissue, while control tests on two livers having no discoverable similar deposits disclosed 0.015 and 0.005 mg. of silica per gram of wet tissue and 0.08 and 0.02 mg. per gram of dry tissue, respectively.

In the spleen the chemical examination revealed 0.2 mg. of silica per gram of wet tissue and 1.40 mg. to each gram of dry tissue, as compared to 0.078 and 0.033 mg. per gram of wet tissue and 0.46 and 0.23 mg. per gram of dry tissue, respectively, in two normal spleens.

The kidney yielded 0.02 mg. of silica per gram of wet tissue and 0.14 mg. per gram of dry tissue, as compared to 0.013, 0.008 and 0.01 mg. per gram of wet tissue and 0.09, 0.04 and 0.05 mg. of silica per gram of dry tissue, respectively, in three normal kidneys.

As a further control, the formaldehyde solution in which the tissue was fixed contained 0.001 mg. of silica per cc. of fluid, as did also freshly made formaldehyde fixing fluid.

It is therefore seen that the content of silica of the tissues concerned was significantly higher in lung, liver and spleen than in normal controls and even in the kidney was higher than in the three controls used.

DISCUSSION

In spite of the difficulties attending positive identification of the dustlike particles in the spleen and liver as silica, there can be no question that it is the same material as seen in the lungs and, further, that there is actual and material damage to spleen and liver tissue by it.

It is interesting to note that the location of deposits in the liver is quite different from that found by Gardner and Cummings[1] in experimental silicosis of the liver, that being periportal in distribution and leading to a portal type of cirrhosis, while here the deposit is of central lobular location, associated prominently with Kupffer cells in the sinusoids and with degeneration and necrosis of liver cells and early fibrosis.

The material is not iron-bearing, it is morphologically like the dust deposits in the lung, the tissues containing it were shown to hold a significant amount of silica by chemical examination, the tissues damaged were in direct relation to the dustlike deposits, and neither the distribution, the tissue change, nor bacteriologic staining indicates that there is associated tuberculosis.

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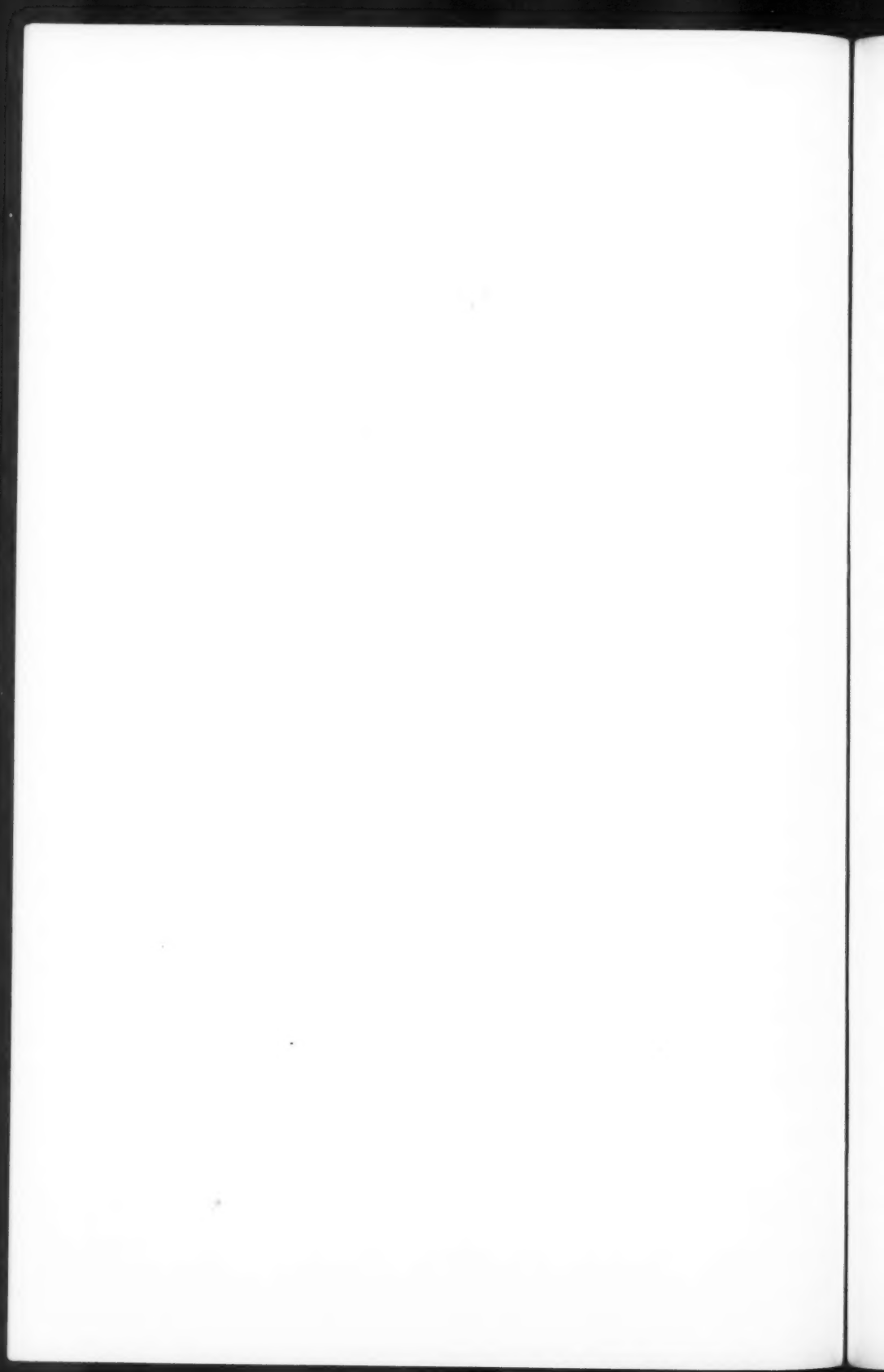
DESCRIPTION OF PLATE

PLATE 56

FIG. 1. Hyaline fibrosis of lung. Silica particles too small to be seen at this magnification. × 67.

FIG. 2. Hyaline fibrous nodule in spleen, with particulate deposit. × 226.

FIG. 3. Deposit of particulate material with cellular degeneration in the liver in silicosis. × 744.
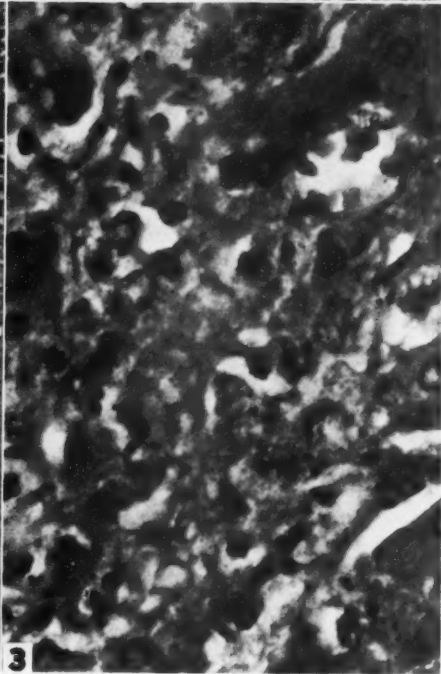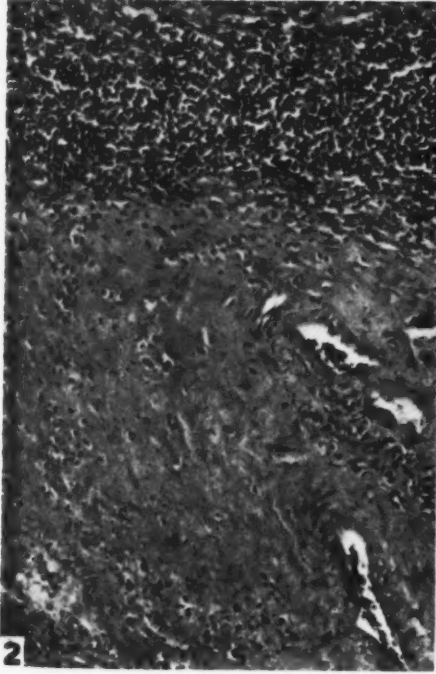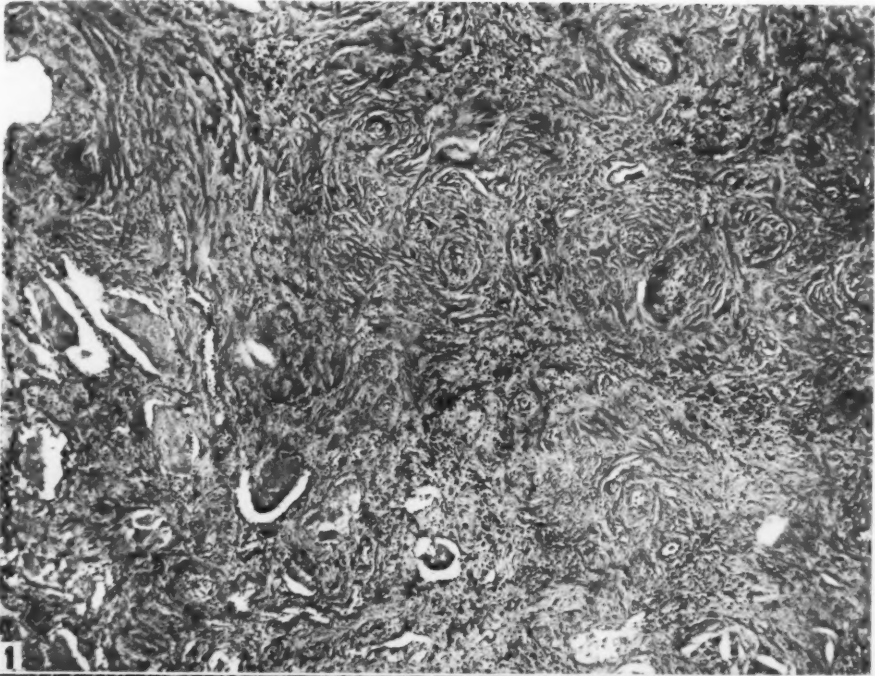
Lynch

Silicosis of Systemic Distribution

EXPERIMENTAL ALLERGIC FOCAL NECROSIS OF THE LIVER*

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Although focal necrosis of the liver is common in infectious and toxic diseases, its nature and etiology are yet not clearly understood. Some believe that this lesion is caused by mechanical ischemia which is secondary to thrombosis of nearby veins and sinusoids, while others think that it is due to the direct action of toxic substances on the hepatic parenchyma without thrombus formation. In the present investigation we have attempted to determine whether thrombosis or direct action of toxic substances, or both, are responsible for the development of focal necrosis of the liver by studying this lesion in hypersensitive animals.

MATERIALS AND METHODS

Most of the animals used in this work were rabbits weighing approximately 2000 gm., although a few guinea pigs were used to determine whether other species might show the same reaction. Injections of 1 cc. of 3 per cent, highly purified, crystalline egg-albumin were made into the radicles of the mesenteric veins, into the marginal ear veins, into the abdominal cavities and directly into the spleens of rabbits which had been previously made hypersensitive by repeated subcutaneous injections of the same protein. From 0.2 cc. to 0.5 cc. of the same protein was injected into the mesenteric veins of hypersensitive guinea pigs. Most of the animals survived this procedure and were sacrificed from 2 hours to several months afterwards. In the earlier part of the work some of them died on the operating table from generalized anaphylactic shock, but it was soon found that this could be prevented if the crystalline egg-albumin was injected very slowly (about 0.15 cc. per minute). A small wedge-shaped piece of liver was taken from each animal at the time of the first operation. After the animals had been sacrificed or had died, the livers, spleens, lungs, hearts and kidneys were examined grossly and microscopically. The tissues were fixed in Zenker's fluid or in a 2 per cent solution of formaldehyde. Sections were stained routinely with hematoxylin and eosin, or with Maximow's hematoxylin-eosin-azure and occasionally with scharlach R and with Mallory's phosphotungstic acid connective tissue stain.

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Approximately 30 animals were given from two to nine injections of crystalline egg-albumin into the mesenteric veins at about 1-week intervals in an attempt to determine whether fibroplastic reactions could be produced in the livers. The animals were then sacrificed and their tissues were studied grossly and microscopically as before.

To determine the specificity of the reaction, one group of rabbits was sensitized with crystalline egg-albumin and later given an injection of horse serum into the mesenteric veins, while another group was sensitized with horse serum and later given an injection of crystalline egg-albumin into the mesenteric veins. All animals of both groups were sacrificed after 24 hours and their tissues were examined grossly and microscopically as above.

OBSERVATIONS

Grossly, the 24-hour lesions were yellowish gray, round, sharply demarcated areas, and measured from pin-point size to 1 mm. in diameter. They were present throughout the liver but were more numerous immediately beneath the capsule.

Microscopically the lesions were focal, either midzonal, peripheral, or almost central in the lobules. True central necrosis, however, was not seen. The lesions varied in size from very small ones, affecting but 20 or 25 cells, to large areas, involving almost the entire lobule. More than one lesion was occasionally present in a lobule and a single lesion sometimes extended to more than one lobule (Fig. 1). Definite foci of beginning coagulative necrosis of the parenchymal cells were seen as early as 8 hours after the injection of the shocking dose of crystalline egg-albumin. By this time, but not before, the sinusoids in the involved areas and many nearby portal veins were markedly congested with polymorphonuclear leukocytes (Fig. 2). Within from 18 to 24 hours most of the affected hepatic cells had lost their integrity; the cords had become homogeneous, granular, eosinophilic masses containing an occasional faded nucleus. The necrotic liver cells were sharply demarcated from the rest of the lobule, and many of the less damaged cells immediately adjacent to the edge of the lesion contained fat droplets (Fig. 3).

Hemorrhage into the necrotic areas was seldom seen, probably because the connective tissue framework of the lobule remained intact despite the massive necrosis of the parenchymal cells (Fig. 4). An occasional thrombus was seen in a portal triad vein near the intralobular lesions, but it should be emphasized that these thrombi were relatively rare in comparison with the large amount of necrotic tissue which was present. After 4 or 5 days many of the necrotic cells and

leukocytes had disintegrated or were in the process of disintegration. After 10 days the necrotic areas had usually disappeared completely, their places having been taken by regenerated liver cells.

Although some animals received as many as nine injections of the antigen into the mesenteric vein, very little increase in the hepatic fibrous tissue was found. Occasionally, however, definite evidence of the experimental production of fibrosis was obtained in livers with normal parenchyma, as shown by biopsy before the injections were begun. Such lesions were located beneath the capsule. The fibrosis was chiefly in the portal areas, but it was also present within the lobule, disrupting the normal lobular architecture (Fig. 5).

None of the control animals that were given injections of crystalline egg-albumin into the mesenteric veins after they were hypersensitized with horse serum showed hepatic changes. Conversely, none of the animals that received horse serum into the radicles of the mesenteric vein after being hypersensitized to crystalline egg-albumin showed liver changes.

Although most of the shocking doses of antigen were injected into the mesenteric veins to insure a constant amount of antigen reaching the liver, massive areas of liver necrosis were present following intra-abdominal, intrasplenic, and peripheral intravenous (marginal ear vein) injections of antigen. The latter route is especially interesting because the antigen had to pass the pulmonary capillary bed before reaching the systemic circulation. It is true that a high percentage of hypersensitive rabbits will die of acute anaphylactic shock following the marginal ear vein injection of the specific antigen if the latter antigen is injected rapidly, but these accidents can usually be avoided if the antigen is injected slowly (0.15 cc. per minute).

The hypersensitive guinea pigs showed massive areas of liver necrosis similar in every respect to the hepatic necrosis observed in rabbits following injection of the specific antigen into the mesenteric vein. This was interesting in view of the general assumption that the lung is the only important site of allergic manifestations in this animal.

No changes were observed in the other tissues (myocardium, lung, kidney, spleen) in any of the animals used in this study.

DISCUSSION

It has been suggested (Mallory,[1] Pearce,[2] Karsner and Aub[3]) that focal necrosis of the liver may be secondary to thrombosis of venules and intralobular sinusoids with a resultant pure mechanical ischemia, and this lesion is occasionally seen at necropsy following propagating thromboses of the portal system. The rarity of this finding in focal

necrosis in human necropsy material indicates that this is not the usual way that this condition is produced. Loeb and Meyers,[4] for example, produced hepatic focal necrosis by the direct action of ether on the parenchymal cells, showing that toxic chemicals alone could produce it without the production of thrombosis. Chemical poisons, however, also are rare in human cases in which focal necrosis of the liver is found. It is possible that liver necroses in cases of idiosyncrasies to drugs and proteins, and sensitizations to bacteria and their products, are secondary to an antigen-antibody union on or within the hepatic cells. Opie[5] has shown that when a foreign protein and its specific antibody are mixed *in vitro*, a precipitate forms which produces inflammation and necrosis when injected into the skin of the normal animal. In passively sensitized animals, necrotic skin lesions form at the site of injection of the specific antigen, but if the antibodies are first precipitated out of the immune serum by treatment with antigen and the supernatant serum is then injected into the normal animal, no local lesion is produced by subsequent intracutaneous injection of antigen (Culbertson[6]). From this it is concluded that the antigen-antibody union is necessary for the production of necrosis. If the necrosis of the liver produced in our experiments (which is similar to that seen in human necropsy material) is secondary to a similar antigen-antibody reaction, one would suspect that this reaction would take place within the liver lobules where the relatively slow circulation and the large surface area allow the antigen the best opportunity to make contact with the antibody on or within the liver cells. Because of the location of the necroses in this area and the relative absence of vascular thrombi, it would seem that the focal hepatic necroses here observed are secondary to a local antigen-antibody reaction.

Rich and Follis,[7] working with normal avascular and vascularized rabbit corneas, concluded that the primary site of action in the Arthus type of hypersensitivity is the vascular endothelium and that tissue death is the result of impairment of nutrition due to vascular damage. We have seen no evidence of sinusoidal endothelial damage in this type of reaction in the liver but realize, of course, that the endothelial cells may be damaged without presenting any morphological changes. However, the absence of edema and hemorrhage from the intralobular lesions is evidence against the presence of endothelial damage.

SUMMARY

1. Massive areas of coagulative necrosis of liver parenchyma were produced by the injection of crystalline egg-albumin into the peritoneal cavity, spleen, or into the mesenteric or marginal ear veins of rabbits previously sensitized to the same protein.

2. Similar hepatic lesions were produced in hypersensitive guinea pigs following the injection of crystalline egg-albumin into the mesenteric veins.

3. Portal fibrosis was occasionally seen following multiple injections into the mesenteric veins of hypersensitive rabbits, but this condition was difficult to produce.

4. Because it was so rarely present in comparison to the large number and large size of the lesions, vascular thrombosis is considered secondary to the massive necrosis of the hepatic cells.

5. No changes in the vascular endothelium and no hemorrhage or edema in the intralobular lesions were observed.

6. Congestion of portal veins and sinusoids by large numbers of polymorphonuclear leukocytes was not observed before areas of beginning coagulative necrosis were present.

7. It would seem, therefore, that the local union of antigen and antibody is responsible for the focal necrosis, and that the manifestations of the response; *i.e.*, coagulative necrosis, polymorphonuclear leukocytic response, and occasional thrombosis of adjacent veins and sinusoids, are all secondary to this antigen-antibody union.

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DESCRIPTION OF PLATES

PLATE 57

FIG. 1. Photomicrograph of a typical liver lesion 24 hours after the injection of 3 per cent crystalline egg-albumin into the mesenteric vein of a hypersensitive rabbit. Massive areas of focal coagulative necrosis and polymorphonuclear leukocytic infiltration (dark areas in photograph) can be seen. × 59.

FIG. 2. A very early lesion (8 hours after the injection of antigen into the mesenteric vein). Beginning focal accumulation of polymorphonuclear leukocytes is shown in the lower left quadrant of the photomicrograph. Thrombosis is absent in nearby veins. × 95.

FIG. 3. A 24-hour lesion showing a typical, well demarcated area of coagulative necrosis of hepatic cells and polymorphonuclear leukocytic infiltration into the affected area. There is an absence of thrombosis in the nearby portal vein as well as of hemorrhage in the lesion. × 174.
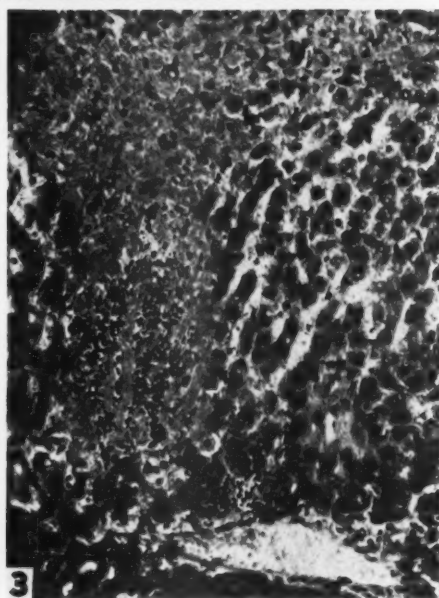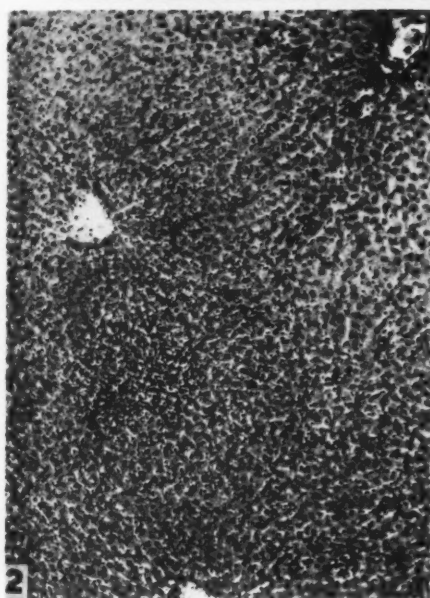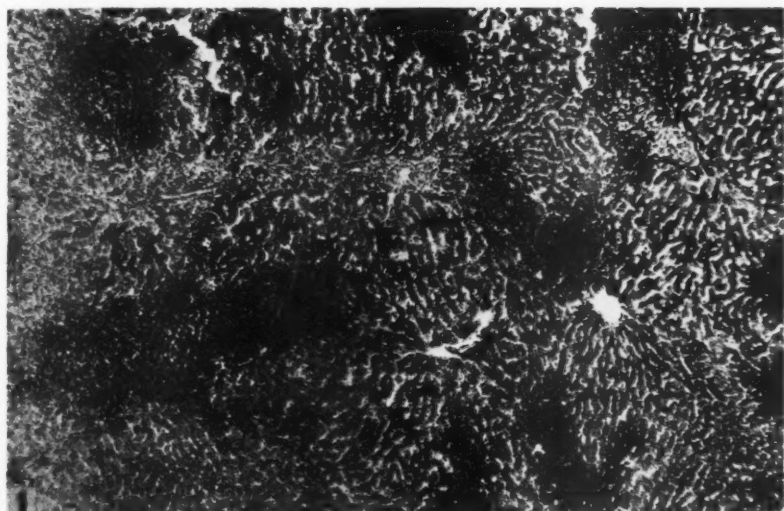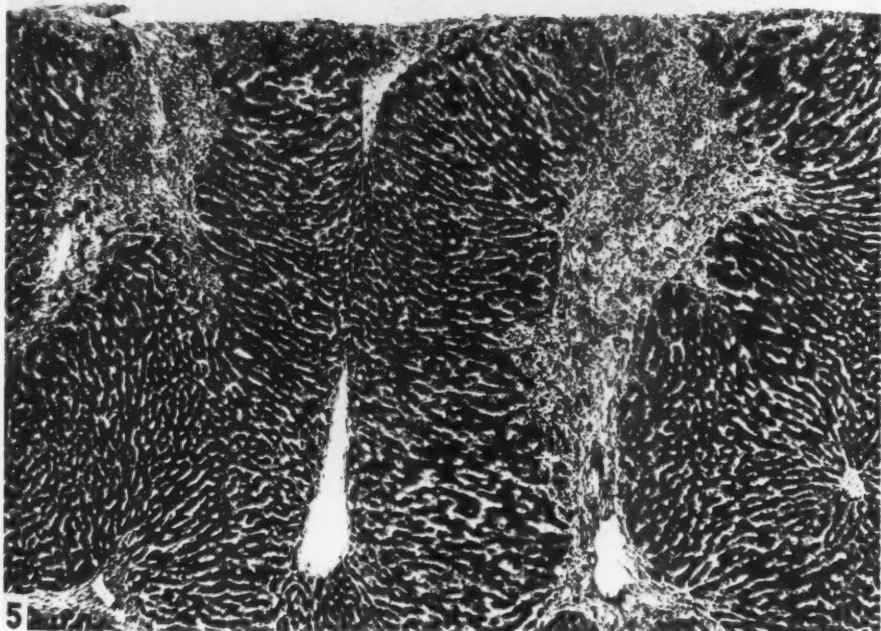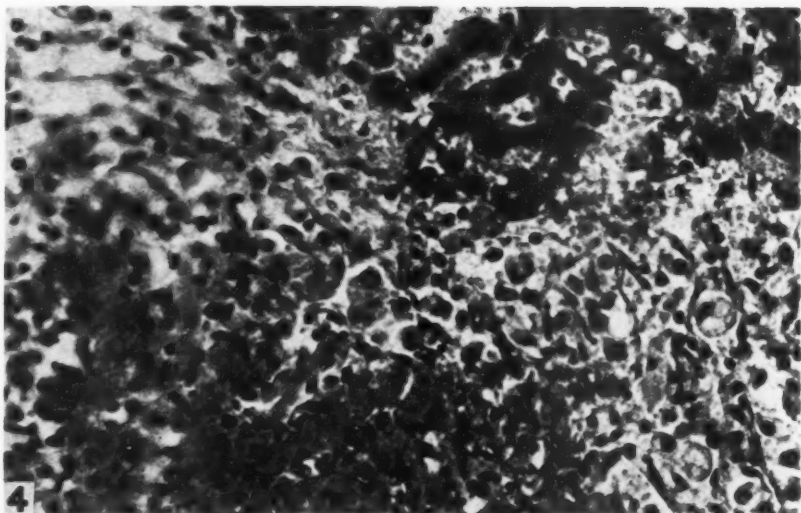
Hartley and Lushbaugh

Allergic Focal Necrosis of the Liver

PLATE 58

FIG. 4. Photomicrograph of a 3-day lesion. Relatively normal liver cells are present in the right upper quadrant while the hepatic cells in the left lower quadrant have undergone coagulative necrosis. The connective tissue framework of the involved area is intact and hemorrhage is absent. There is also a beginning infiltration of inflammatory cells into the affected area and an appearance of fat droplets in some of the less involved cells adjacent to the lesion. $\times$ 329.

FIG. 5. An area of sub-capsular portal fibrosis. This rabbit had had 5 mesenteric intravenous injections, each of 1 cc. of 3 per cent crystalline egg-albumin, over a period of about 8 weeks. $\times$ 66.

Hartley and Lushbaugh   Allergic Focal Necrosis of the Liver
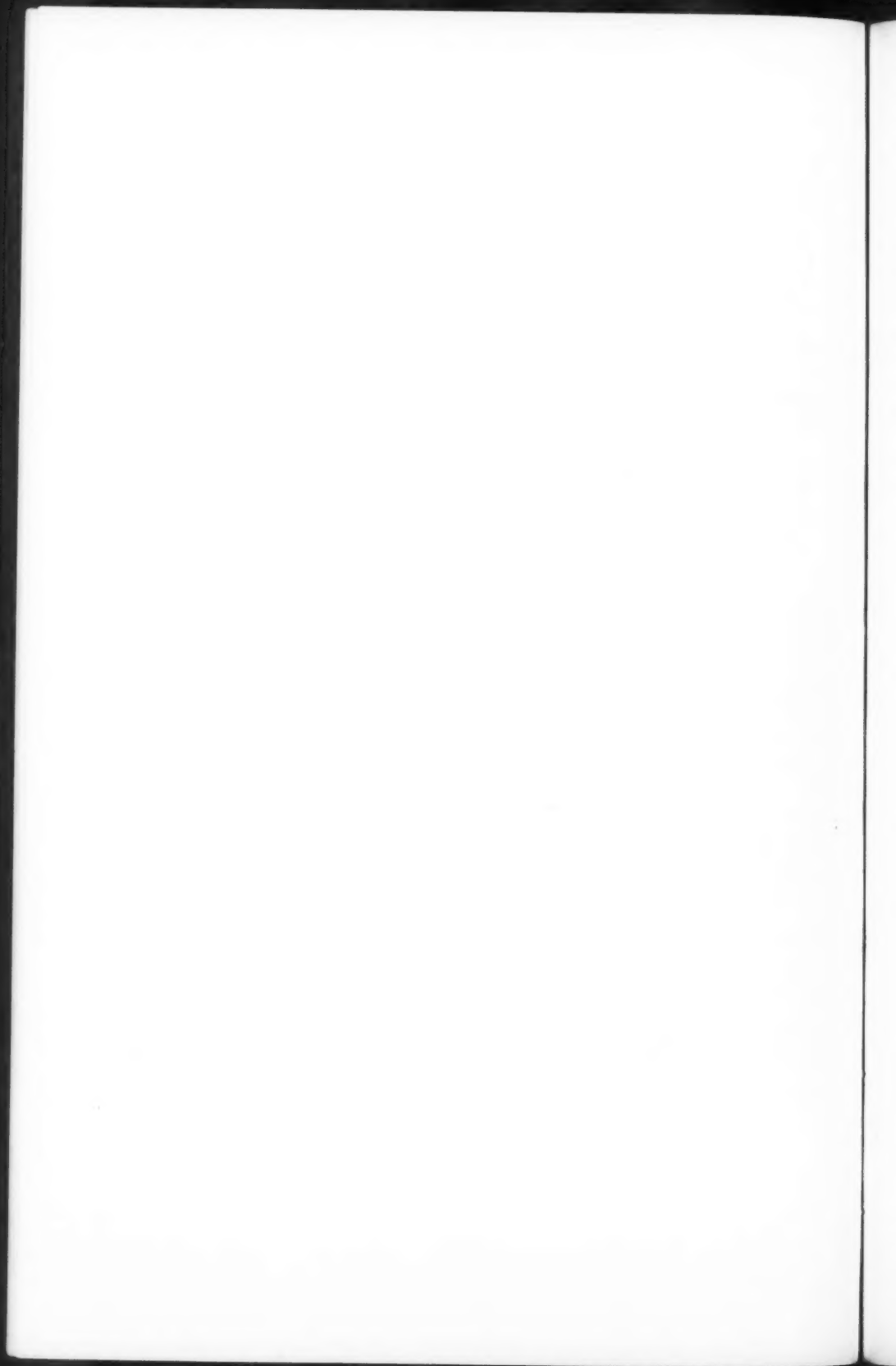
PROTEOLYTIC DIGESTION OF RED AND WHITE
BLOOD CORPUSCLES IN THE SPLEEN*

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Enlargement of the spleen occurring in response to infection, usually designated as acute splenic tumor, is in some instances associated with injury or destruction of red blood corpuscles, and phagocytosis of red cells is conspicuous in the pulp of the spleen. These changes are well illustrated by the acute splenic tumor of typhoid fever; the spleen is soft and may be diffluent.

When washed erythrocytes of the pigeon were injected by Addison[1] into the blood stream of rabbits, phagocytosis of these cells by mononuclear phagocytes proceeded very actively in the sinuses of the pulp of the spleen and phagocytes were largest about 16 hours after injection. Perfusion of the spleen facilitated the histological study of the changes in the pulp.

Wehrle,[2] in this laboratory, studied the fate of the red and white blood corpuscles of the rabbit introduced through an ear vein into the circulating blood of animals of the same species. There was active phagocytosis, and intracellular digestion of both erythrocytes and granulocytes was seen in the splenic cords and was somewhat less in the sinuses. Perfusion of the spleen washed the red corpuscles and lymphocytes from both cords and sinuses, but, as actual count showed, left mononuclear phagocytes and granulocytes almost undisturbed. Phagocytosis of erythrocytes and of granulocytes, determined by counts of the number of mononuclear cells that had ingested these cells, increased, reached a maximum between 16 and 18 hours after injection and then diminished.

Experiments have been undertaken to determine if there is increase of proteolytic activity of splenic tissue corresponding with the phagocytosis of red and white blood cells that occurs following their introduction into the blood stream.

Methods

Young rabbits weighing usually from 1700 to 2300 gm. were used. Blood corpuscles, obtained by intracardiac puncture with aseptic precautions, were washed three times with normal salt solution and then brought to the original volume of the blood. Twenty cc. of this suspension of red and white cells were injected into the lateral ear vein of

each rabbit and the animals were killed at different intervals after the administration of the cells. The spleen was immediately perfused with salt solution passed through a cannula inserted into the aorta at the level of the celiac axis. After ligation of all arteries except the splenic, the perfusion fluid escaped from the splenic vein. Perfusion was continued for 10 to 20 minutes until the color of the spleen changed from purple to orange-pink. The method was, in general, the same as that used by Wehrle,[2] but the spleen was perfused with a pressure of 60 cm. of water instead of 120 cm.

A weighed quantity of the perfused spleen ground to a pulp was incubated with pure casein at 37° C. and proteolytic digestion was measured by increase in nonprotein nitrogen. The nonprotein nitrogen content of samples of the mixture removed at different intervals after the beginning of incubation was determined. Protein was precipitated with trichloracetic acid and removed by filtration. The nitrogen content of the filtrate was determined by micro-Kjeldahl analysis. The initial nonprotein nitrogen present in the mixture of spleen and casein was determined in a sample removed as soon as the mixture was prepared, and when this figure was subtracted from that obtained from samples removed at different intervals after incubation at 37° C., the remainder was the measure of the digestion that had occurred. The nonprotein nitrogen in the mixture before incubation in all instances varied from 4 to 6 mg. per gram.

Digestion occurred in the presence of physiological salt solution or of Sorenson's phosphate buffer at pH 6.3, 6.7, 7.0, or 7.4, the hydrogen ion concentration being checked colorimetrically with bromthymol blue. Toluene was used as a bacteriostatic agent. The mixture was made up in the following proportions: 1 gm. spleen, 0.5 gm. casein and 50 cc. of buffer solution, the actual volume being adjusted to the quantity of spleen that was available. Preliminary experiments had shown that 1 gm. of spleen incubated alone and thus allowed to undergo autolysis gave values varying from 8 to 13 mg. of nonprotein nitrogen. It is convenient to express the results of digestion in milligrams of nonprotein nitrogen obtained by the action of 1 gm. of spleen. Casein was incubated alone at hydrogen ion concentration varying from pH 6.3 to 7.4 and there was no increase of nonprotein nitrogen during 30 hours of incubation. The casein that was used contained 13 per cent nitrogen, 0.5 gm. containing 68 mg. of nitrogen. It may be assumed that figures for nonprotein nitrogen in excess of 8 to 13 mg. per 1 gm. of spleen are the result of digestion of the casein present in the mixture.

All animals were autopsied and routine microscopical sections made from lung, liver and kidney to exclude the presence of disease, and no pathological change was found in any of the animals. Microscopical

sections of the perfused spleens were examined to determine the activity of phagocytosis. The results of these observations were in accord with those of Wehrle,[2] but actual counts of phagocytes were not made.

In preliminary experiments proteolysis was measured after 24 hours of digestion and the results are shown in Table I. The figure representing nonprotein nitrogen of the mixture before incubation has been subtracted from that obtained after digestion.

TABLE I

Proteolysis Produced by Normal Spleen and by Spleens of Animals That Had Received Blood Corpuscles

Rabbit no.	Blood corpuscles injected into vein	pH	Increase of nonprotein nitrogen after 24 hours at 37° C.
7	None	No buffer	9 mg. N. P. N. per gm. of spleen
		pH 6.3	26 mg. N. P. N. per gm. of spleen
		pH 6.3	21 mg. N. P. N. per gm. of spleen
		pH 6.7	21 mg. N. P. N. per gm. of spleen
8	None	pH 6.7	21 mg. N. P. N. per gm. of spleen
		pH 7.4	4 mg. N. P. N. per gm. of spleen
9	Corpuscles from 20 cc. of blood given 17 hours before animal was killed	pH 6.7	51 mg. N. P. N. per gm. of spleen
10	Corpuscles from 20 cc. of blood given 17 hours before animal was killed	pH 6.7	48 mg. N. P. N. per gm. of spleen
11	Corpuscles from 20 cc. of blood given 17 hours before animal was killed	pH 6.7	53 mg. N. P. N. per gm. of spleen
22	Same but with no perfusion of spleen	pH 6.7	17 mg. N. P. N. per gm. of spleen

When no buffer was used to maintain the original hydrogen ion concentration of the mixture, digestion was inhibited, presumably by the accumulation of products of digestion. With pH of 6.3 and of 6.7, digestion during 24 hours produced by normal spleen was active, but with greater alkalinity, namely, with pH 7.4, scant digestion occurred. The proteolytic activity of splenic tissue 17 hours after the introduction of blood corpuscles into the circulating blood was much greater than that of normal spleen.

Proteolysis caused by spleen with no perfusion was found to be much less than that caused by perfused splenic tissue. In Table II, representing several similar observations, digestion at pH 6.7 caused by tissues from a spleen with no perfusion, removed from an animal that had received blood corpuscles 17 hours before death, is compared with that caused by a perfused spleen from a similarly treated animal. Digestion is evidently inhibited by the presence of blood serum within the spleen and proceeds more actively when this is removed by perfusion.

The introduction of granulocytes into the circulating blood, their accumulation in the spleen, and phagocytosis by mononuclear phagocytes within the pulp and sinuses suggested the possibility that the

weak leukoprotease of the granulocytes of rabbits might have a part in
the proteolysis demonstrated by the foregoing experiments, but the
scant proteolysis in the presence of an alkaline medium, namely, pH
7.4 (Table I), showed that proteolysis by leukoprotease was an insig-
nificant factor in the increased proteolysis caused by splenic tissue
following intravenous injection of blood corpuscles. This increased
digestion occurred under conditions that favored the activity of the

TABLE II

Spleen with no perfusion (no. 22)		Spleen with perfusion (no. 28)	
Hours of digestion	Increase of nonprotein nitrogen in mg.	Hours of digestion	Increase of nonprotein nitrogen in mg.
24	17	24	50
50	19	48	77
72	19	72	82
Nonprotein nitrogen before digestion was 4 mg.		Nonprotein nitrogen before digestion was 4 mg.	

proteolytic enzyme found in histiocytes that have assumed the function
of macrophages.[3]

In subsequent experiments the proteolytic action of splenic tissue
on casein at different intervals after intravenous injection of blood
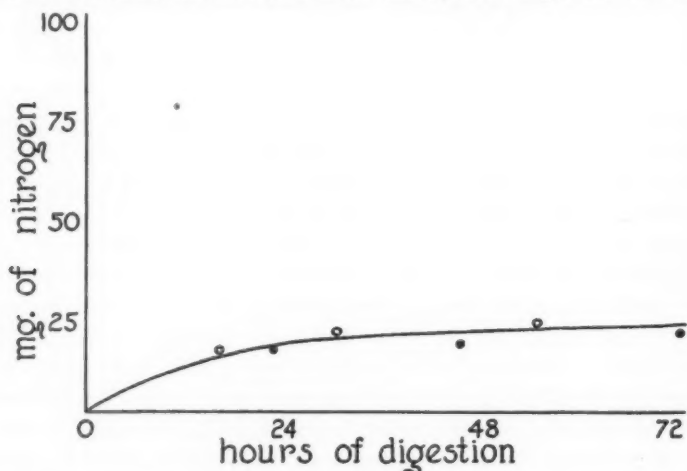
TEXT-FIGURE 1. A composite graph showing digestion produced at pH 6.7 by 1
gm. of splenic tissue from two normal animals.

corpuscles was measured after increasing periods of digestion up to
72 hours. Digestion was measured with hydrogen ion concentration
adjusted by buffer solutions to pH 6.7, 7.0 and 7.4, and was found
to be most active at pH 6.7. No digestion occurred during 30 hours
at pH 7.4.

Text-figure 1 is a composite curve prepared to show the results of di-

gestion at pH 6.7 caused by splenic tissue of two normal animals. There
has been no increase of proteolysis in the period after 30 hours of
incubation, and the non-coagulable nitrogen has risen to a maximum
of less than 25 mg. of nitrogen.

Text-Figures 2 to 5 show, after different periods, the activity of
digestion caused by perfused splenic tissue removed 6, 17, 24 and 48
hours after injection of blood corpuscles into the circulating blood.
Within 6 hours after injection of corpuscles the activity of proteolysis
has increased greatly above normal and has maintained this level for
at least 24 hours. After 48 hours the curve of digestion has returned
to normal.

TABLE III

Duration of digestion	pH	Increase of nonprotein nitrogen*
24 hours	6.7	28 mg.
72 hours	6.7	36 mg.

* Nonprotein nitrogen before digestion was 4 mg.

TABLE IV

Duration of digestion	pH	Increase of nonprotein nitrogen*
24 hours	6.7	50 mg.
48 hours	6.7	71 mg.
72 hours	6.7	71 mg.
24 hours	7.0	15 mg.
48 hours	7.0	24 mg.
72 hours	7.0	47 mg.

* Nonprotein nitrogen before digestion was 4 mg.

Text-Figure 6 shows the activity of digestion at increasing intervals
after intravenous injection of blood corpuscles under conditions that
were found favorable for proteolysis, that is, at pH 6.7, and after a
uniform time interval of 24 hours of digestion. It is noteworthy that
the curve rises promptly and maintains an almost uniform level from
6 to 24 hours, being highest at 17 hours, and then falls to a normal
level 48 hours after injection of blood corpuscles.

Two experiments were performed to determine the effect of repeated
injection of blood corpuscles on the proteolytic activity of splenic tissue.

A rabbit (no. 26), weighing 2000 gm., received on 3 successive days
washed blood corpuscles from 20 cc. of blood. When the animal was
killed 12 hours after the last injection, the spleen measured 42 by 9 by
5 mm. Digestion by the perfused spleen in the presence of casein at
pH 6.7 is shown in Table III.

A rabbit weighing 2700 gm. received washed blood corpuscles from
20 cc. of blood on 4 successive days and was killed 24 hours after the
last injection. The spleen measured 55 by 14 by 8 mm. The progress
of digestion with casein caused by the perfused spleen is shown in
Table IV.

Digestion by splenic tissue after repeated intravenous injections of blood corpuscles has been more active than that produced by normal splenic tissue, but proteolytic activity of the same quantity of tissue has not increased above a maximum represented by that following a
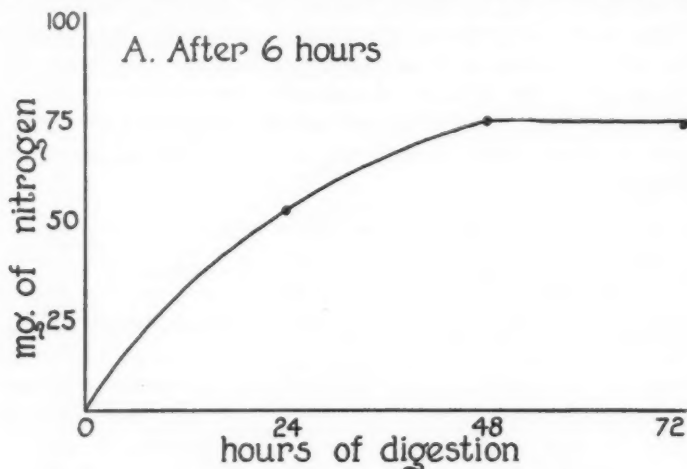
TEXT-FIGURE 2. Graph showing the activity of proteolytic digestion at pH 6.7 caused by 1 gm. of splenic tissue (A) 6 hours after intravenous injection of blood corpuscles.
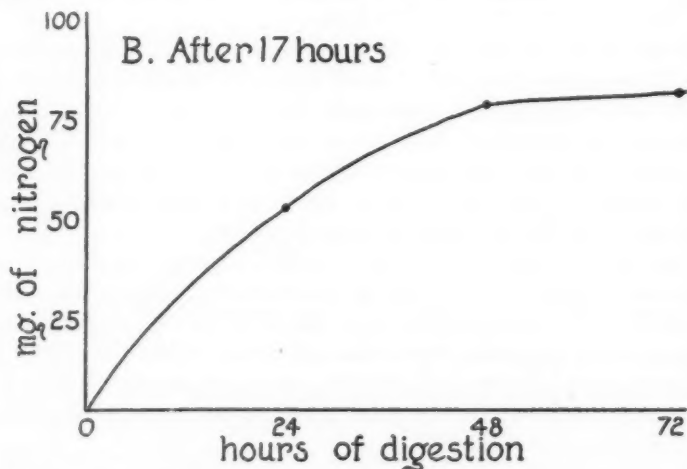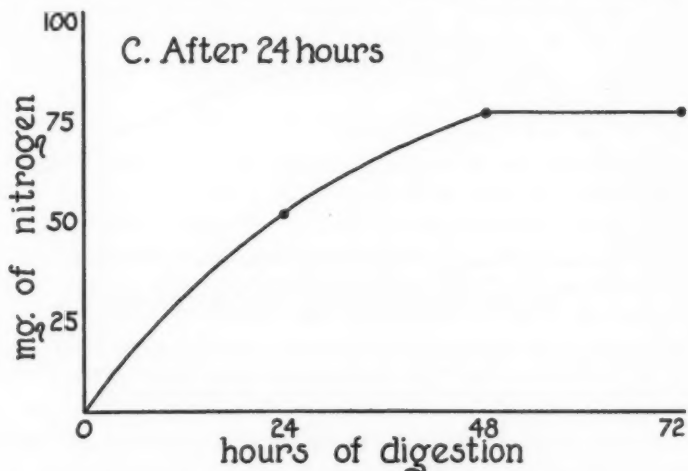

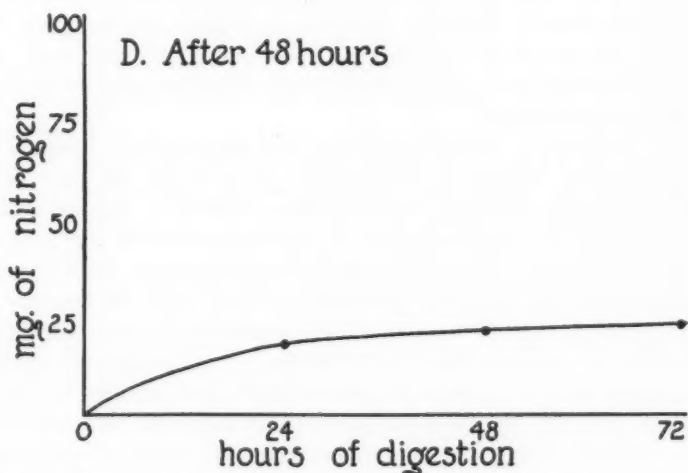TEXT-FIGURE 3. As in Text-Figure 2: (B) 17 hours after injection.

single injection of blood corpuscles (Table II). It is noteworthy that the volume of the spleen in the second experiment had increased to considerably above normal.

DISCUSSION

A single injection of washed corpuscles, Wehrle [2] found, increased the size of the spleen from a normal average of 43 by 6 by 2 mm. to 53 by 9.4 by 3 mm. Rous and Oliver [4] injected 10 cc. of citrated whole
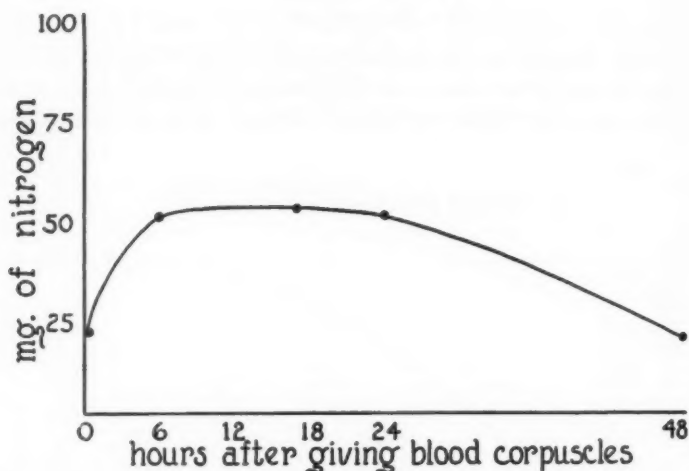

TEXT-FIGURE 4. As in Text-Figure 2: (C) 24 hours after injection.

TEXT-FIGURE 5. As in Text-Figure 2: (D) 48 hours after injection.

blood into the circulation of rabbits 6 days each week for several months. After 3 or 4 weeks the spleen became much enlarged and was made turgid by phagocytes crowded with red cells and cellular débris.

TEXT-FIGURE 6. Graph showing the activity of proteolytic digestion during 24 hours at pH 6.7, caused by 1 gm. of splenic tissue removed from rabbits at different intervals after introduction of an excess of blood corpuscles into the circulating blood.

After months of this plethora the spleen was diminished in size and the sinuses contained masses of hemosiderin.

The changes in the spleen found in our experiments are analogous to those of the red type of acute splenic tumor described by Evans [5] and distinguished by him from a gray type of acute splenic tumor, the former being associated with typhoid fever and related infections and the latter, he believed, with pneumococcal, staphylococcal, streptococcal and other infections.

It is noteworthy that phagocytosis and intracellular digestion of granulocytes as well as of erythrocytes occur within the pulp cords and sinuses of the spleen of normal rabbits (Wehrle [2]). Normal splenic tissue contains an enzyme capable of splitting protein in the presence of an acid medium. Its activity can be measured only after blood is removed from the spleen by perfusion. Parallel with the increase of phagocytosis of washed red and white corpuscles following the introduction of an excess of these cells into the circulating blood, there is increased proteolytic activity of splenic tissue, measured by its ability to bring about the digestion of casein. This increased proteolytic activity reaches a high level soon after injection of blood corpuscles, maintains this elevated level from 6 to 24 hours after injection of corpuscles and has disappeared 48 hours after the injection.

The enzyme that causes proteolysis in the spleen acts best in the presence of hydrogen ion concentration of pH 6.7 and is almost com-

pletely inhibited by an alkaline reaction of pH 7.4. In these characters it resembles the proteolytic enzyme that is present in the mononuclear phagocytes of an inflammatory exudate (Opie[3]) and in the macrophages that accumulate in lymph nodes adjacent to the site of inflammation. It is distinguishable from the leukoprotease of the granulocytes, which are ingested by the phagocytes of the spleen, by its failure to act in the presence of an alkaline medium.

Summary and Conclusions

Parallel with increased phagocytosis of red and white blood corpuscles in the spleen after injection of these cells into the circulating blood of rabbits, a measured quantity of splenic tissue causes increased proteolysis *in vitro*. Increased proteolysis is caused by a proteolytic enzyme present in the macrophages of the organ and measurable only after blood has been washed from the splenic tissue by perfusion.

This proteolytic activity is maintained at a high level from 6 to 24 hours following introduction of an excess of corpuscles into the blood and has returned to normal after 48 hours.

Parenteral digestion is a significant function of the spleen and proceeds with increased activity in some forms of "acute splenic tumor."

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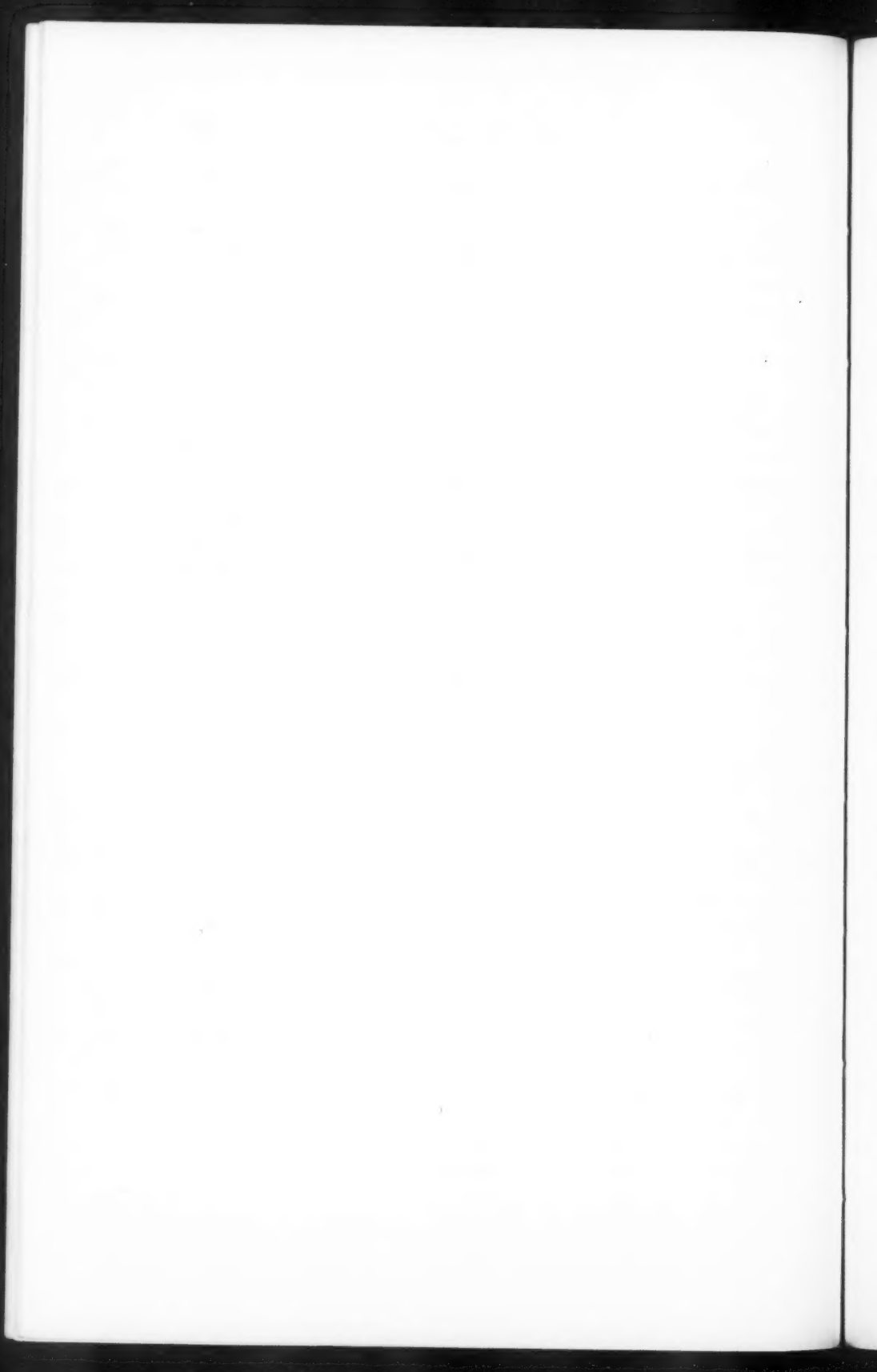
SUBAORTIC STENOSIS*

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The less frequent cardiac malformations are always of pathologic interest. The example of subaortic stenosis encountered by us is especially interesting because a correct diagnosis was made during life.

REPORT OF CASE

History. R. H. K., a white male, 26 years old, entered the Multnomah County Hospital on March 23, 1935, complaining of cough, weakness and a swollen jaw. At the age of 10 years the patient had scarlet fever. Following this he learned that he had a "blow" in the heart and found he could not run as fast as his playmates. For the past 2 years he had not felt well, had tired very easily and had noted an increased pallor. Three months before admission sweats and chills developed, and for the last 3 to 4 months he had been coughing up about an ounce of sticky sputum a day. Following extraction of several carious teeth 1 month earlier, several small, painful, hemorrhagic spots developed at the tips of the fingers. There was no history of chest pain, nocturia or dependent edema. At the age of 16, after hard physical exertion, he was cyanotic for a few hours.

Physical Examination. Pulse, 110 per minute; respirations, 24; temperature, 101.0° F.; blood pressure 102/80. The important physical findings were as follows: The gums were swollen and bled very easily. There was no cyanosis. The heart was enlarged chiefly to the left. There was no lower sternal propulsion noted. A definite systolic thrill, detected over the second right interspace, was transmitted to the great vessels of the neck and down the left side of the sternum. The pulmonary second sound was not felt. A purely systolic murmur, most evident in the second right interspace, was transmitted to the vessels of the neck and down across the sternum to the left. The heart rhythm was regular. The aortic second sound had a clicking character. It was short, sharp and sounded close to the ear. The peripheral pulse was small and poorly sustained. The spleen was enlarged to palpation.

Laboratory Data. On entrance the following determinations were made: hemoglobin, 38.2 per cent; red blood cells, 2,490,000; color index, 0.72; white blood cells, 7,050; polymorphonuclear leukocytes, 61 per cent; lymphocytes, 21 per cent; staff cells, 12 per cent; sedimentation rate, 110 and 156 mm. (15 and 45 minutes). Evidence of a moderate degree of erythropoiesis was observed in the blood film. The Kolmer and Kahn reactions were negative. Urine: specific gravity, 1,010; yellow; acid, and cloudy with occasional hyaline and granular casts. Pus cells ranged from ++ to +++ and erythrocytes were listed as + in one of two examinations. Streptococci and gram-negative bacilli were found in the blood cultures but were not identified further. Interpretation of the electrocardiographic tracings was "questionable coronary disease."

Clinical Diagnosis. (Dr. Maurice F. Gourley.) Rheumatic aortic endocarditis, probably with considerable calcification; superimposed subacute bacterial endocarditis; subaortic stenosis.

After a progressive downward course, the patient died 42 days after hospitaliza-
tion. The temperature varied from 100° to 103° F., occasionally rising to 104°,
while the pulse ranged from 90 to 110 per min.

POSTMORTEM EXAMINATION

The postmortem examination was performed 15 hours after death.
The body appeared slightly undernourished and pallor was evident.
Aside from edema of the ankles there were no external changes. About
150 cc. of transudate were present in the peritoneal cavity. Approxi-
mately a liter of similar fluid filled the right pleural cavity while the left
contained about 200 cc. The spleen weighed 540 gm. and contained a
large, yellow, anemic infarct. A branch of the splenic artery supplying
this portion of the spleen was occluded by a septic thrombotic embolus
in which there were plates of lime salt like those in the aortic valve.
The kidneys were swollen, soft and varied from yellow to bright red.
Microscopically, they showed typical subacute proliferative glomerulo-
nephritis with occasional partially infarcted glomeruli attributable to
embolic occlusion. The other salient findings were encountered only in
the heart.

Heart

Gross Examination. There were approximately 100 cc. of clear fluid
in the pericardial sac. The heart had a diameter of 14.5 cm. and
weighed 450 gm. The left ventricle, from the base to the apex, meas-
ured 10 cm. The thickness of the myocardium on the left and at the
base was 2.2 cm., and 0.7 cm. at the apex. The chamber seemed rela-
tively small in comparison to the dilated right cavity. The wall of the
right ventricle was 0.7 cm. in thickness. One cm. inferior to the base of
the aortic valve ring there was an annular, shelflike stenotic ring con-
sisting of pale, grayish white, smooth, firm tissue covered everywhere
by intact endocardium. This band was located 0.6 cm. caudal to the
right posterior aortic valve cusp, but approximated and fused with the
bases of the anterior and left posterior leaflets (Fig. 1). The ridgelike
structure extended 0.6 cm. into the ventricular cavity and was not more
than 2 mm. thick at any point. The endocardium between the posterior
aortic valve cusps and the subaortic ring was smooth, glistening, opaque
and slightly irregular. Here was found an endocardial pocket, or
pseudo-valve, with an orifice measuring 0.6 by 0.2 cm. Its ostium lay
close to, and opened toward, the base of the left posterior aortic valve
cusp. Through it a probe could be passed towards the infra-aortic ring
for a distance of 0.5 cm. The ring had a circumference of 2.5 cm. It
extended well onto the ventricular surface of the aortic leaflet of the
mitral valve but lacked 0.8 cm. of reaching the junction with its
chordae tendineae. The anterior leaflet of the aortic valve was normal

save for slight fenestration along its free edge. The right posterior leaflet, however, was quite large, measuring 2.5 cm. in breadth at its commissural attachments and 1.5 cm. in height. The left posterior leaflet was 1.7 cm. in breadth. The large right posterior cusp was thickened and indurated. Adherent to the ventricular surface of the half nearest the left posterior leaflet was a firm, grayish yellow to pink nodular vegetation measuring 0.6 by 0.9 cm. Adherent to the aortic surface of this same cusp (Fig. 1) was a moderate amount of granular, pinkish yellow material. Similar excrescences were present on the ventricular aspect of the left posterior cusp, mainly involving the half of the valve near the commissure separating the two posterior leaflets. The aortic valve ring measured 6 cm. in circumference. Over the intima of the ascending aorta about the orifice of the left coronary artery there was a yellowish to grayish pink sessile elevation measuring 0.6 to 0.7 cm.; otherwise the inner surface of the aorta was smooth and glistening. Except for the extension of the subaortic annular ring onto the mitral, this structure was negative. The mitral valve ring measured 8.0 cm. in circumference. A few small focal areas of fibrosis were seen in the papillary muscles of the mitral valve. There were no associated anomalies other than a slight anatomical patency of the foramen ovale.

Microscopic Examination. The free border of the subaortic band consisted of hyalinized fibrous connective tissue which was largely covered by intact, flattened endothelium. At the junction of the ring with the myocardium there was a single focus of lymphocytes. The endothelium along the inner edge of the aortic valve and over the ventricular surface was replaced by a thin zone of polymorphonuclear leukocytes. Near the base of the valve the tissue was thicker, hyalinized, contained more polymorphonuclear leukocytes caught in fibrin, and showed areas of calcification. Bacterial stains revealed moderate numbers of gram-positive cocci in the central portion of the vegetation and along the free ventricular border of the valve. Sections of the myocardium proper showed a few minute foci of recent necrosis, unaccompanied by inflammation, and slight hypertrophy of the muscle cells. No structures that could be interpreted as active or healed Aschoff bodies were observed.

COMMENT

Judging from the slight degree of cardiac hypertrophy (450 gm.), maximum thickness of the left ventricular wall (2.2 cm.) and the slight dilatation of the left chambers, the subaortic annular stenotic ring and thickened aortic cusps failed to produce much obstruction or incompetence, yet formed a sufficient barrier to the passage of blood to evoke

the systolic thrill and murmur detected clinically. Another fact indic-
ative of diminution in output was the small and poorly sustained periph-
eral pulse. Clinically, the clear-cut aortic second sound was
interpreted as evidence that this valve was functioning properly. The
diagnosis of subaortic stenosis was based upon the evidence of aortic ob-
struction with retention of the second aortic tone. The presence of a
small endocardial "bird's nest" pocket between the aortic valve and the
subaortic ring is an additional point against full competency of the
valve. Such endocardial pockets are usually found in association with
valvular insufficiency, and, as in this instance, open in the direction of
the regurgitation. The fact that the nidus lay superior to the subaortic
ring and was somewhat recessed makes it difficult to believe that it
could have been responsible for the clinical observations or that it con-
tributed appreciably to the changes observed at necropsy. Before the
annular ring was sectioned at necropsy the right posterior aortic valve
leaflet was found to be directly in line with the probable flow of blood
coming from the ventricle. Inasmuch as the largest vegetation was
present here, it further strengthens the view that trauma plays a de-
cided etiologic rôle in acquired valvular affections. Clinically it was
thought that the bacterial endocarditis was secondary to carious teeth.
Both may have been responsible for the associated glomerulonephritis.
This latter condition, together with the blood-stream infection, ade-
quately explains the intense hypochromic anemia. The pulmonary
edema was thought to be secondary to cardiac failure.

DISCUSSION

The subject of subaortic stenosis has been thoroughly studied by
Abbott [1] who, in 1927, was able to catalogue twenty-six instances.
Enzer,[2] in 1927, described an anomalous subaortic bicuspid valve, lo-
cated 3 cm. below the aortic valve. This anomalous valve had chordae
tendineae attached to a papillary muscle. This is not a clear-cut in-
stance of subaortic stenosis and is not included in the total list of cases.
Sternberg,[3] in 1930, described two cases of this condition. One occurred
in a woman, 27 years old, in whom heart disease had been clinically
recognized since the age of 5. At the age of 19 years a diagnosis of
aortic stenosis was made. The illness that led to her death was a septic
sore throat. At necropsy a subaortic annular ring was found 6 to 7 mm.
below the aortic valve ring. This fibrous band was only 2 mm. in height
and appeared smooth and white. The ventricles and auricles were
slightly dilated; no other congenital or acquired lesions were present.
The second case was that of a man, 77 years old, who had arterio-
sclerosis, pulmonary emphysema and, later, obstructive icterus. In this
heart a small, irregular, fibrosed band extended from the lower part of

the membranous interventricular septum onto the aortic leaflet of the mitral valve. The aortic valve showed extensive calcification. Sergent, Launay and Imbert,[4] in 1932, reported an example of congenital sub-aortic stenosis occurring in a male, 21 years old, which was complicated by an acute aortic bacterial endocarditis. In this case the subaortic ridge rose 1 cm. above the level of the interventricular wall.

A recent report,[5] of a boy, aged 14 years, with congenital subaortic stenosis and narrowing of the brachiocephalic venous trunks, is a clinical study and makes no factual mention of subaortic stenosis. This instance consequently is not included in the present review.

Dormanns,[6] in 1939, reported an instance of subaortic stenosis in a male adolescent, 16 years and 9 months of age. This person had had measles as a child. On entering school a systolic heart murmur was discovered accidentally. Subsequent examinations revealed this murmur to have become more intense and audible over the entire cardiac area. Nevertheless he was active in athletics and had no apparent difficulty in various competitive sports. One morning, without significant previous exertion, he suddenly collapsed and died within 2 hours. Autopsy, performed 72 hours after death, revealed a heart weighing 375 gm., which had an anomalous subaortic ring and thickened aortic valve leaflets. The collar-shaped subaortic ring lay 7 mm. below the aortic valve ring and was wedge-shaped in cross section, measuring 3 mm. in width at its base and 3 mm. in height. It was located 21 mm. below the upper border of the aortic valve and 8 cm. from the apex of the left ventricle. The aortic valve ring was 5.5 cm. in circumference and all three of its leaflets were thick, whitish and opaque. The left ventricle was enlarged and dilated. The anomalous ring was formed of fibrous connective tissue lying beneath an intact endocardium. There was no significant cellular infiltration. In the aortic leaflets an increase of fibrous connective tissue was apparent as well as considerable myxomatous tissue beneath the endocardium over the free edge of the valve. Here, as in the stenotic ring, significant cellular infiltration was absent. The myocardium showed no changes. Stating that the examination revealed no evidence of fetal or post-fetal endocarditis, Dormanns explained the subaortic stenosis and the changes in the aortic valve as due to a complicated arrest of development. No other cardiac abnormalities were found.

We feel that our case is a true example of subaortic stenosis, bringing the apparent total to thirty-one.

As to the pathogenesis of subaortic stenosis we can do no better than to quote Abbott:[1] "Two groups are to be differentiated, (a) those in which the lesion is associated with other anomalies, in which case a true arrest of development with survival of the bulbus cordis in the aortic

vestibule has occurred, and (b) a subaortic stenosis of inflammatory origin due to an infective process setting in [in] early postnatal or late prenatal life." While not susceptible of proof, the appearance of cardiac symptoms following scarlet fever at the age of 10 years, together with the lack of associated cardiac anomalies or evidences of arrested development, are points much in favor of an acquired lesion due to inflammation. Abbott [7] further emphasized that the lesion "does not in itself interfere with cardiac function but is nevertheless of serious significance on account of the fact that it is liable to become the seat of a subacute infective process." Cyanosis, clubbing of the fingers and evidence of aortic valvular stenosis are usually absent. In our case the subaortic ridge escaped but the aortic valve and the aortic intima were the seats of bacterial infection.

SUMMARY

The example of subaortic stenosis forming the basis of this report brings the total of reported cases to thirty-one. There is evidence suggesting, but not proving, that in this instance the original process was acquired. Bacterial infection was present on the aortic valve but not on the subaortic ridge when the patient died at the age of 26 years. The clinical and pathologic aspects of this case are presented and the incidence and characteristics of subaortic stenosis are discussed.

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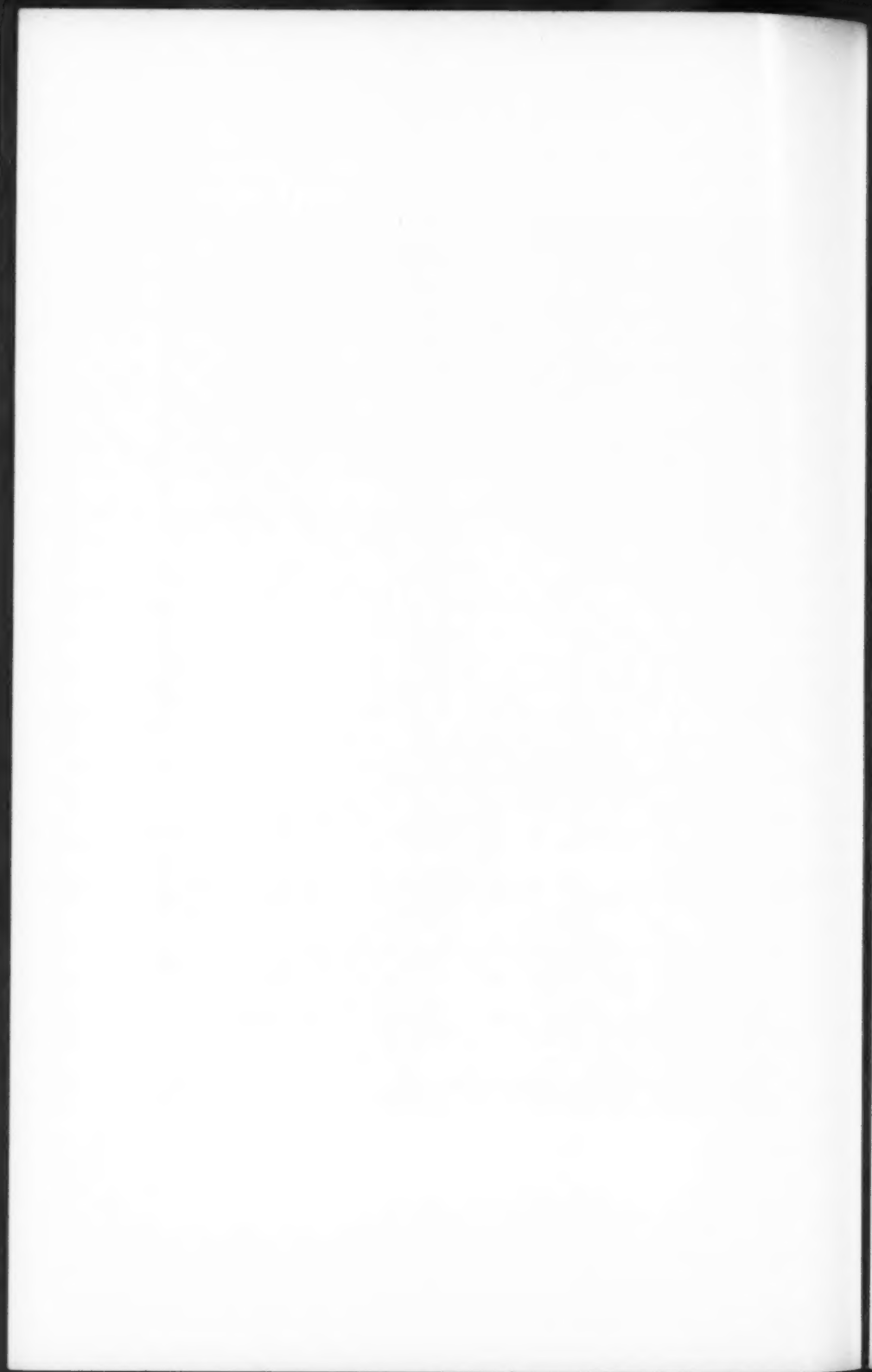
DESCRIPTION OF PLATE

PLATE 59

FIG. 1. Photograph of left ventricle and aortic valve showing clearly the shelflike, annular stenotic ring below the aortic valve. The dark, oval, ragged areas on the right posterior aortic valve cusp and aortic intima are ulcerative vegetations.
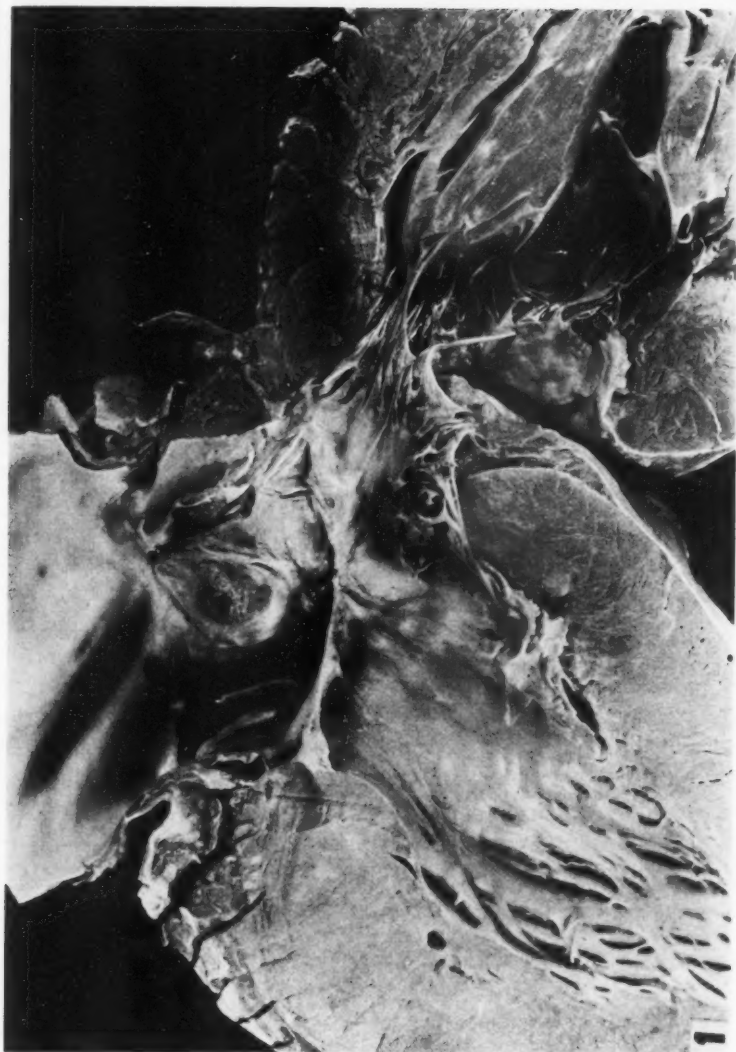
Mason and Hunter   Subaortic Stenosis

MICROFILARIAL GRANULOMATA OF THE SPLEEN*

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Filarial lesions have been noticed very rarely in autopsies performed in Bombay. In an analysis of over 5,000 autopsies performed at the King Edward VII Memorial Hospital, Bombay, no such lesions could be discovered. However, experience derived from the out-patient department and the wards of the hospital seemed to suggest that filarial infection was not uncommon. It was well recognized that the presence of a filarial worm in the body did not necessarily mean that a pathological lesion resulted in the tissues. The female worms lying in the deep lymphatics did not produce changes sufficiently marked to be detected unless an autopsy was carried out with a view to detecting the localization of the worms in the different parts of the body. If lesions such as elephantiasis, lymphadenopathy or chylous effusions in serous sacs were present, their filarial nature was immediately recognized. During the course of the last 2 years we have encountered lesions in the spleen which could be readily detected macroscopically and which appeared to be microfilarial in origin.

The fecundated female worm lying in the deeper lymphatics gives rise, periodically, to broods of larvae which enter the general circulation to be distributed to different parts of the body. They have been observed in the capillaries of many organs such as the liver, spleen, kidneys and lungs. Manson,[1] the discoverer of filarial disease, stated: "The healthy fully formed microfilariae—that is to say the embryonic filariae which by means of the microscope we see in the blood are, so far as we can tell, particularly harmless." Being harmless it was believed that they did not produce any lesions in the body. Our observations now present evidence that microfilariae are not always innocuous and that they are capable of producing lesions in deep organs such as the spleen.

MATERIAL

Our material consisted of eleven specimens of spleen, obtained at autopsy, from patients in the King Edward VII Memorial Hospital, Bombay, in which multiple nodules were found in that viscus. Table I gives the duration of hospitalization and the clinical and postmortem diagnoses of the cases. It shows that death was due to injury in

more than half of these patients and that their stay in the hospital before death varied from a few hours to 3 or 4 days. The significance of these findings is discussed later.

OBSERVATIONS

Macroscopical Appearances of the Lesions in the Spleen

The spleens bearing the lesions were largely normal in size and weight. Table II gives the age of each patient and the weight of the spleen. The spleen of case no. 4 was abnormally large because of an accompanying lobar pneumonia. The resulting gray splenic tumor gave rise to the increase in weight and not the nodules which are to be described.

TABLE I

No.	Duration of hospitalization	Clinical diagnosis	Postmortem diagnosis
1.	2 days	Paraplegia	Transverse myelitis
2.	½ hour	Head injury	Fracture, skull
3.	1½ hours	Multiple fractures	Multiple fractures; shock
4.	Few minutes	Lobar pneumonia	Lobar pneumonia
5.	4 days	Head injury	Multiple fractures
6.	1 day	Catarrhal jaundice	Subacute atrophy, liver
7.	10 hours	Uraemia	Chronic nephritis; uraemia
8.	Few minutes	General peritonitis	Duodenal perforation
9.	Few hours	Multiple fractures	Multiple fractures
10.	Few hours	Head injury	Fracture, skull
11.	Few hours	Fracture and dislocation of spine	Multiple fractures

TABLE II

Serial No.	Age of the patient (years)	Sex	Weight of the spleen (gm.)	Size of the organ
1.	65	M	150	Normal
2.	40	M	90	Normal
3.	40	M	120	Normal
4.	35	M	540	Enlarged
5.	50	M	60	Small
6.	25	F	180	Normal
7.	50	M	...	Little larger
8.	15	M	...	Normal
9.	6	F	90	Enlarged
10.	8	M	45	Normal
11.	50	F	50	Smaller

In these spleens nodules could be felt at the surface, as seen in Figure 1. They were smooth and rose a little above the surface. They were a little firmer than the rest of the splenic tissue and their size varied from 2 to 25 mm. Usually they were multiple, but occasionally only a single nodule was present. On the cut surface the nodules presented a reddish or reddish brown appearance quite distinct from the chocolate colour of the splenic parenchyma. They were sharply circumscribed and occasionally two or three of them merged into one

large nodule. In a preserved organ the nodules stood out on the cut surface (Fig. 2). Although first considered endotheliomata, a detailed study of their histology soon showed that a certain proportion of them were characterized by marked eosinophilic infiltration. This led to an intensive search for a parasitic cause and resulted in finding microfilariae in every one of them.

Histology of the Lesions

In histological preparations each nodule was seen as an area with a closer texture than the surrounding splenic tissue. It was separated from the latter by a slitlike space (Fig. 4), due to the fact that the nodule, being of a closer texture, did not shrink to the same extent as the surrounding splenic tissue in the process of preparation of the section. That may also account for the fact that the nodules projected on the surface in a preserved specimen. The margin of the lesion was well defined and usually presented a convex outline. Although different nodules showed minor variations, in general they presented two main types of reaction. In one hyperplasia of the reticulo-endothelial cells was a pronounced feature, while in the other the sinuses showed marked dilatation and engorgement with red cells. Irrespective of the type of reaction, the lesions invariably showed eosinophilic infiltration, either focal and intense, or diffusely scattered (Fig. 5). The eosinophil content of the nodules was much greater than in the surrounding normal splenic tissue. In addition to eosinophils, the nodules contained a varying number of giant cells and lymphocytes. The giant cells in some sections were quite conspicuous, while in others they were found only after special search. They were four or five times the size of a lymphocyte and on an average showed 5 to 8 nuclei. The lymphocytes were an inconspicuous element in the nodules. In some nodules the fibrous connective tissue was diffusely increased, as shown by Mallory's stain. In others it was the reticular tissue which had undergone hyperplasia. This was shown in sections stained by Foot's method (Fig. 6). Where the reticular tissue was increased the fibrous connective tissue generally did not show an increase. The nodules did not contain any malpighian bodies or large-sized blood vessels. Apparently they derived their blood supply from capillaries and sinusoids. It may thus be seen that the histological structure of the lesions was that of a granuloma.

Microfilariae

Every one of these lesions, irrespective of the extent of eosinophilic infiltration, showed the presence of microfilariae on cut section. The

number of microfilariae varied considerably. In some, every field
under a one-sixth inch objective showed a fair number, while in others
a prolonged search had to be carried out to locate a single microfilaria.
Their number could not be associated with any particular histological
appearance.

The microfilariae in cut section were distinctive. Most of them
showed a clear space about the segment (Fig. 7). The walls of the
body of the microfilaria were parallel and the interior contained a
series of granules. Unless these were present it was difficult to identify
the structure as a microfilaria. In our preparations we were not able
to obtain an entire organism, the longest segment seen being 108 by
5 μ (Fig. 8). From histological sections alone it was difficult to
identify the species of the microfilaria,* which might be either of the
two species (*Wuchereria bancrofti* and *W. malayi*) known to produce
infection in India. As seen in Figure 7, the granules in these micro-
filariae were large, separate and easy to count. Before attempting to
ascertain the species it would be necessary to obtain entire specimens
of microfilariae by teasing fresh nodules.

Microfilariae in Other Organs

In those cases in which microfilariae were present in nodules in the
spleen, search was made for them in other organs (Table III). Un-
fortunately, tissues from all the organs were not available for study.
Microfilariae were found to be present in the capillaries of the heart,

TABLE III

The Occurrence of Microfilariae in Various Organs

Serial no.	Spleen (no. of microfilariae seen)	Other organs from which sections were available	
		Microfilariae present	Microfilariae absent
1.	Large number	Kidney, heart and pancreas	......
2.	Few	......	Liver
3.	Large number	......	......
4.	Large number	Kidney	Gallbladder and liver
5.	Few	......	Kidney, lung, liver and heart
6.	Few (long)	Kidney and lung	Liver, heart and pancreas
7.	Moderate	Kidney (large number), liver, brain, testis and epididymis	......
8.	Moderate	Liver	......
9.	Moderate	......	Brain and ovary
10.	Moderate	Lung (large number)	......
11.	Moderate	......	Liver and heart

lungs, kidneys, liver, brain, testis and epididymis without any reac-
tion around them. In one case sections from kidney, lung, liver and
heart were available but microfilariae were not found. However, in

* The microfilaria have since been identified as *Wuchereria bancrofti*.

five cases from which the kidneys were available for histological examination, four showed microfilariae in their substance, caught in the glomerular capillaries. In one case numerous glomeruli appeared larger than normal and all of these contained microfilariae. This patient had died of chronic nephritis and uraemia. Before death the urine contained albumin and casts. Histological examination of the kidneys gave evidence of chronic nephritis, but the glomeruli in which microfilariae were caught did not show any evidence of such inflammatory changes as exudate in the Bowman's capsular space or cellular infiltration inside or outside of the glomerular tuft. Whether microfilariae in a large number of glomeruli contributed mechanically to the final outcome of the case is difficult to determine.

Microfilariae Ante Mortem

In only one of our cases were the microfilariae detected in the blood during routine examination: a patient with deep jaundice who died in the hospital as a result of subacute atrophy of the liver and cholaemia. No filarial lesions were discovered at autopsy and subsequent microscopical examination of the liver and other organs, except the spleen, did not show microfilariae.

DISCUSSION

We have been unable to discover any reference in the literature to lesions similar to those described in this paper. The nearest description of microfilarial lesions which we were able to trace was by Bonne.[2] He has described an infestation of splenic tissue with microfilariae in a Javanese male, who was killed in a motor accident. Microfilariae were discovered during a routine histological examination of the internal organs. Nothing was known about the presence of microfilariae in the patient's blood. The other internal organs did not show microfilariae. The spleen had reacted with an extraordinary degree of eosinophilia and some peculiar giant cells were noted surrounding the terminal ends of the larvae. The extreme eosinophilia, for which no other explanation was available, was believed to have some connection with the presence of microfilariae. The process was considered as either an undescribed phase in the life cycle of one of the common filarial species or a manifestation of an unusual filarial parasite. Altogether this condition was in many ways comparable to an eosinophilic microfilarial manifestation in lymph glands described by Meyers and Kouwenaar.[3]

The microscopical characters of the lesions described by Bonne[2] more or less resemble those described by us. From the summary of

his article it appears that he did not notice gross nodular lesions such as those described by us. His finding of microfilariae in the sections was accidental, while we were led to search the nodules because of the presence of foci of eosinophilic infiltration. He was not able to find microfilariae in other organs. In some of our cases microfilariae were seen in organs other than the spleen.

We have considered the possibility that in the lesions described by us the presence of microfilariae was accidental, and that the nodules were due to some other etiological factor. This appears to us unreasonable in view of the fact that the microfilariae appeared to be concentrated only in the nodules, whereas in the surrounding splenic tissue they were rare and scattered, and were lying in the lumina of the blood vessels. In the nodules the reaction was unmistakably directed towards a limitation and possibly towards destruction of the numerous larvae that were present. In some of our sections microfilariae are seen in various stages of disintegration, a finding which indicates that their presence was not accidental.

In the case described by Bonne,[2] and in many of those described by us, death was accidental. The duration of stay in the hospital was very short, varying from a few minutes to 3 or 4 days. This might be significant because it was only in such cases that lesions of a granulomatous nature which would ultimately become patches of fibrosis were likely to be encountered. This, we believe, was probably one of the reasons why the lesions described by us were not detected before. The spleen is richly endowed with reticulo-endothelial tissue which plays an important rôle in phagocytosis and the destruction of any particulate material. It is quite conceivable that the nodules are an attempt on the part of the splenic tissue to get rid of dead microfilariae. From the staining character of the microfilariae in sections, not all of them appeared to be dead, although there was no doubt that some were in various stages of disintegration. The presence of living microfilariae in the circulating blood in one of our cases militates against the hypothesis that all the microfilariae were dead and that this was one of the methods by which they were being disposed of from the body.

As previously stated, the nodules varied greatly in regard to their content of microfilaria (Table III). The number of microfilariae present probably depends on the age of the lesion. In an old and fibrosed lesion one would expect to find a small number of microfilariae, while in a young lesion they are likely to be numerous. The histological characters of the lesions supported this view.

SUMMARY

Multiple gross nodular lesions seen in the spleens of 11 patients are described. Microscopical examination showed them to contain microfilariae in every case. Evidence has been presented to show that these granulomata are microfilarial in origin.

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DESCRIPTION OF PLATES

PLATE 60

3

5 m.m.

4

/ m.m.
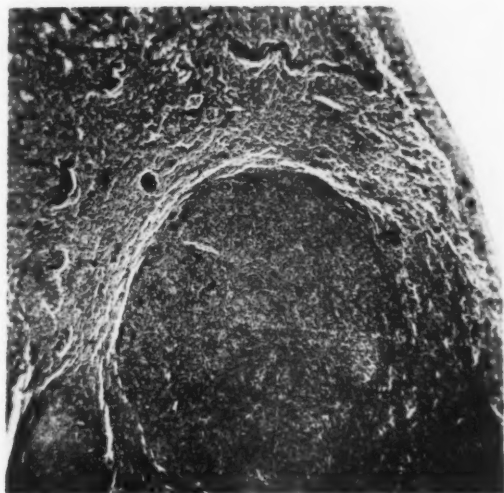
Dhayagude and Amin

Microfilarial Granulomata of the Spleen

PLATE 61

FIG. 5. A photomicrograph with oil immersion lens taken to show the eosinophile cell infiltration around the microfilaria which is seen in the centre. The cells with dark staining cytoplasm are the eosinophiles.

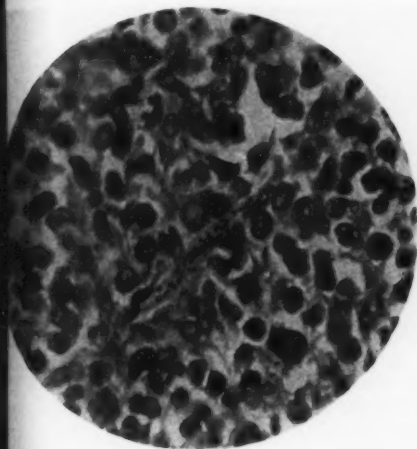FIG. 6. Shows the increase in the reticular tissue as demonstrated by Foot's method of staining.

FIG. 7. Shows two microfilariae in cut sections embedded in a large amount of inflammatory cellular reaction.

FIG. 8. Shows a large segment of a microfilaria in which the characters of the granules are well demonstrated.
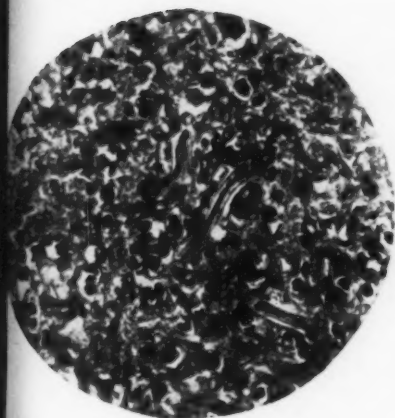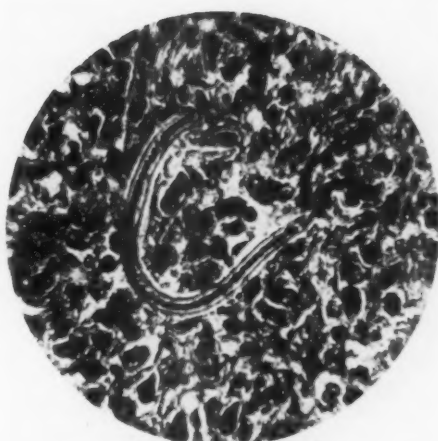
haya

6

8

20 μ

100 μ

50 μ

50 μ